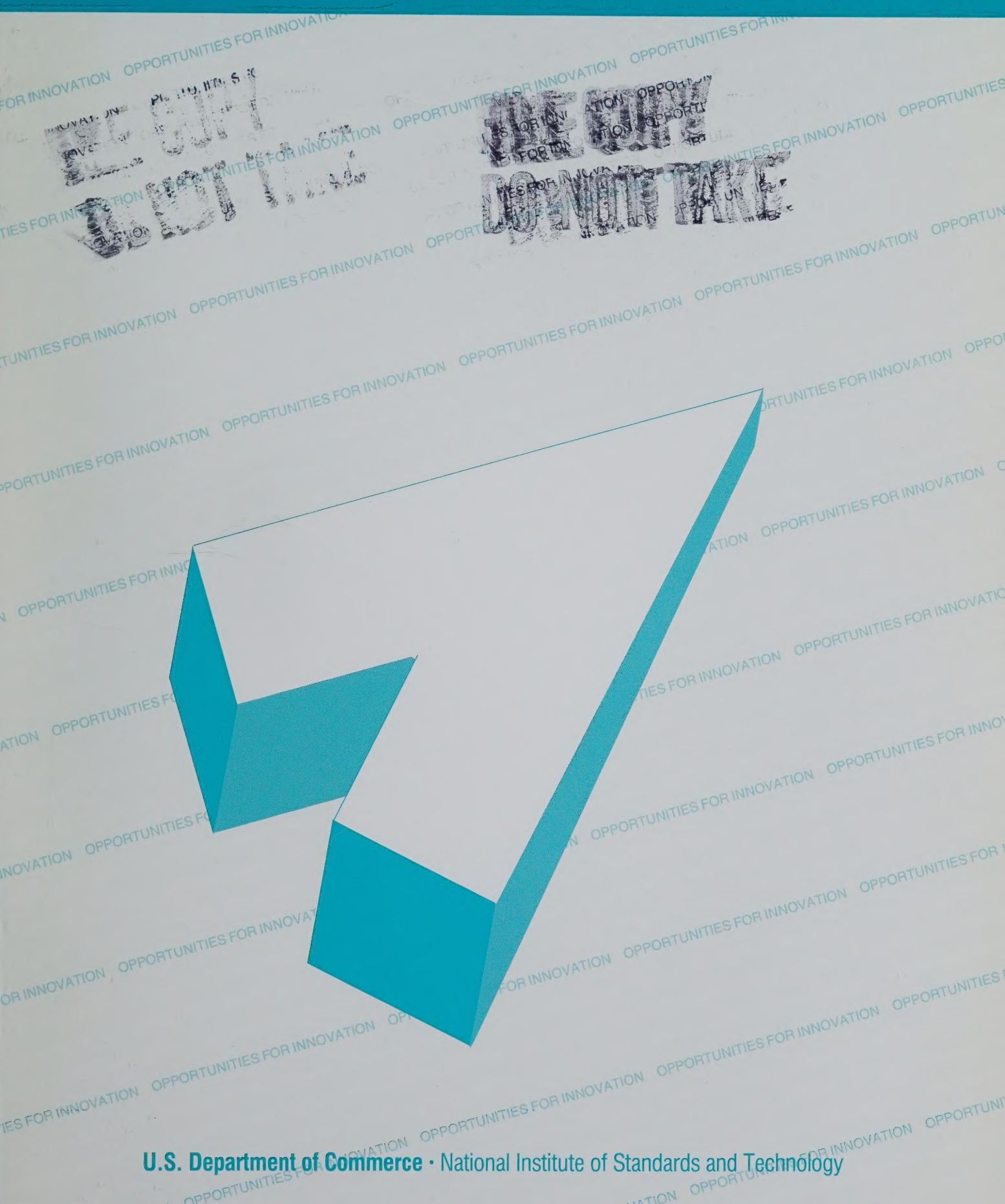


CHEMICAL AND BIOLOGICAL SENSORS



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Opportunities for Innovation: Chemical and Biological Sensors

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FOREWORD

In the world of competitive business, the phrase "technology transfer" has come to mean movement of technological information either from universities or from federal laboratories to industrial companies capable of translating this information into a commercial product or process. However, it is intercompany communication, formal and informal, oral and written, that accounts for most of the technological information absorbed and utilized by an innovating company. About two thirds of all technological innovations that result in the commercialization of a new process or new product originate outside the company (Thomas J. Allen: "Managing the Flow of Technology" MIT Press, 1977). Among the written documentation transmitting technological information, unpublished company reports are, by far, the most important source of information. These reports may have originated in companies that are in a position of either a user or a supplier with respect to the receiving company. Oral communication of innovative technological ideas is about half as frequent as printed information. Person-to-person contacts are most valuable in providing supplemental information as follow-up to written documents. The participation in this process of information diffusion and absorption of technological information requires a significant investment of staff time. In some companies, engineers and scientists spend more than twenty five percent of their time acquiring and processing information.

The majority of small companies do not participate in this process fully because of their limited human and material resources. Therefore, these small firms that are manufacturing a single product or a limited line of products are often unaware of the many opportunities that exist outside their main line of business. And yet, because small companies are frequently endowed with a great deal of enthusiasm, entrepreneurial spirit, motivation, and intellectual capital, they could increase their output of new products given the opportunity.

The series of NIST monographs called "Opportunities for Innovation" has been conceived with the small, technology based company in mind. Each monograph provides the technical staff of a small firm with a "multicompany" report on the best opportunities for new business endeavors in a technology driven market.

Each monograph forms a backbone for a series of regional workshops keyed to the relevant topic. The workshop concept is to add the elements of oral communication and person-to-person contact to form a comprehensive technology transfer mechanism. Further, the workshops provide a forum for the formation of intercorporate partnerships and strategic alliances.

Each chapter of the monograph, written by an expert in the field, focuses on those aspects of a specific technological discipline where new scientific advances hold promise of early translation into technological innovations. The authors point out the gaps and short-comings in the state-of-the-art that prevent the commercialization of the art and they explain those aspects of recent scientific and engineering development that need further work.

The monographs are written for engineering and technical people. Those in the business and finance communities who have sufficient technical background will also benefit from studying these monographs. The first monograph in the series of "Opportunities for Innovation" dealt with polymer composites, a fast evolving technology, finding wide applications in the automobile, aerospace, and consumer goods markets.

The present volume in this series, "Chemical and Biological Sensors," continues in the same vein. The field is full of fledgling, mostly privately held companies looking for markets in the chemical and processing industries, in the automotive and power generating industries, and in the health care industry. At the same time, users of industrial sensors have expressed disappointment in the lack of interest shown by sensor manufacturers in their specific needs.

The field for new sensors is wide open. The U.S. in-vitro diagnostic sensor market is expected to grow at a rate of 8 to 10% annually over the next 5 years to exceed \$6.5 billion in 1986. In the same year, the value of all sensors used in engine management (i.e. emission control and fuel efficiency) is expected to reach half a billion dollars. Other opportunities exist in the food processing industry, in pharmaceuticals, and in environmental control. Clearly, there is a need for more well-planned contacts between sensor manufacturers and those who use them. The "Opportunities for Innovation" program is designed to assist in this process.

John W. Lyons
Director, NIST

PREFACE

As with the other monographs in the NIST series, the objective in preparing this monograph on sensors was to encourage the industrial competitiveness of small businesses in the United States. The consensus is that the combined sensor market is huge and our motivation for this monograph was to get more small U.S. companies interested in the potential locked up in this rapidly growing area. The bio- and chemical sensor area represents an ideal one for small businesses due to the fragmentary and niche-oriented nature of the markets these sensors often cover. There are, however, some signs that the United States might become an early loser in the sensor manufacturing arena of these devices. Indeed, in Germany and Japan, the governments have sponsored major efforts to commercialize sensors. Also, the short-term profit requirements by U.S. companies are typically less important in Japan, and smaller markets are not sneered at. Characteristically, in the two main manufacturing countries in the world, Japan and Germany, it seems to have been recognized instinctively that the micromanufacturing technologies utilized to make sensors constitute a new way of looking at manufacturing in general. These micromanufacturing technologies are leading to a whole new generation of advanced microstructures that will have an impact on all of industry in the coming years.

The individual chapters in this monograph are meant to review the most important chemical and biosensors today and to discuss associated problems before these devices can become commercial successes. The choice of type of sensors discussed was mainly dictated by their commercial potential, the market need, and the advent of new technologies in these sensing areas. We have also included one general chapter outlining the opportunities and challenges for sensor development and another one giving the readers some insight in market size and the availability of capital for funding work in this area. Recognized experts on these topics were chosen to author the different chapters.

One of the most crucial aspects in commercialization of all sensors is the introduction of batch manufacturing techniques. We have attempted to educate our readers first on micromanufacturing or micro-fabrication technologies involved in making bio- and chemical sensors. These manufacturing technologies are often borrowed from the IC industry but do not require the huge up-front investment in capital equipment. Indeed for many of the sensors discussed in this monograph rather rudimentary equipment, often second-hand equipment from the IC industry, is sufficient. Wafer size is of little importance in this industry and most of the time we do not need the line width required for state-of-the-art IC manufacturing. Consequently, the equipment in the sensor industry can be used much longer than the same equipment in the IC industry. All of these factors make an entry in the market quite possible even for smaller companies.

To ensure technical accuracy and clarity, each chapter in this monograph was reviewed by selected personnel from Teknekron Sensor Development Corporation (TSDC). We are especially indebted to Allison Andersen for her patience in editing the manuscript.

In addition to the authors, credit is due to Dr. J. J. (Jarda) Ulbrecht of the National Institute of Standards and Technology who, with an ever critical eye, was always looking for the highest quality product.

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Application of Micromachining in Solid-State Chemical Sensors

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The application of micromachining technologies in the construction of new types of chemical sensors and the potential market for these sensors is reviewed.

Two major categories of chemical sensors are discussed: i.e., solid-state gas sensors and electrochemical sensors. In the solid-state gas-sensor category, we are detailing issues concerning semiconductor sensors for reducing agents, humidity sensors, the solid-electrolyte air/fuel ratio combustion sensor, and the catalytic sensor. The choice of these specific sensors was dictated by their market importance. In the technical sections, the most pressing technological barriers that need to be overcome for wider acceptance of the sensor product are detailed, and possible solutions are suggested. We will show that these solutions are often to be found in the application of microfabrication technology.

The technical discussion is followed by a market analysis for these same sensor products. In the market section we are making a distinction between current sensor applications and emerging market applications. Often the emerging market applications will only become possible after micromachining technology has been properly applied to existing products.

In summary, we will learn in this chapter that the application of micromachining technology to the construction of sensors not only solves many of the technological issues but it also makes the products more acceptable for a wider market.

Key words: electrochemical; micromachining; sensor; sensor markets; solid state.

1. Introduction

Solid-state sensors that measure the universal physical parameters such as pressure, position, height, acceleration, and temperature have been commercially successful. Less success has been attained by solid-state sensors designed to measure important chemical species such as protons (pH), O₂, CO₂, H₂S, CO, propane, and glucose, to mention just a few. There is no doubt, however, that we can look forward to a rapidly increasing use of solid-state sensors, as the results of current research lead to their improvement. Furthermore, the use of microfabrication technologies in the manufacturing of sensors will result in considerable cost savings compared with the cost of more complex analytical equipment, such as gas chromatographs.

This savings will further stimulate sensors' wider acceptance.

In microfabrication, one borrows from batch-type IC manufacturing processes and applies those processes to non-IC devices, such as sensors. Microfabrication often involves not only Si but also ceramics, metals, and organics. Therefore, we prefer a new terminology—*advanced microstructure technology*, that explicitly refers to a new way of manufacturing. This manufacturing method borrows technologies from the semiconductor industry but is not limited to the materials or devices one typically deals with in that industry.

In this chapter, I shall review the technical aspects of solid-state sensors and review their poten-

tial market. We separate our discussions of solid-state gas sensors from our review of electrochemical type sensors. We try to indicate for all sensors discussed, the advantages advanced microstructure technology can bring. For example, we all know that the major disadvantages of most solid-state chemical gas sensors are lack of stability, lack of reproducibility, and lack of selectivity, as well as insufficient sensitivity for certain purposes. Micro-fabricated arrays of sensors feeding a microcircuit can in principle overcome many of the above disadvantages while still retaining a reasonably low cost.

In general, I want to explore the reasons why solid-state chemical sensors have not yet become a commonplace item in our daily lives. I also suggest possible directions for future research and development.

One important recognition in terms of the current market share of chemical sensors is that they have often been oversold. Rather than addressing specific market needs, the new chemical sensors were frequently promoted as a generic solution to all sensing problems. The emphasis was often on selling a certain new technology rather than focusing on solving a specific sensing problem. I have found that there are a lot of specific market needs where new solid-state chemical sensors can be introduced as useful tools, but they are far from a generic solution at this stage. Those specific markets are very significant in size. American industry should look at advanced microstructure technology not only as a way of regaining competitiveness in solid-state chemical sensors but as an approach to manufacturing new products in general.

2. Solid-State Gas Sensors

Presently there are four types of solid-state gas sensors in substantial use commercially:

- The semiconductor sensor for reducing agents
- The humidity sensor
- The solid electrolyte air/fuel ratio combustion sensor
- The catalytic sensor for the low-cost measurement of combustible gases, especially where higher concentrations (say the order of ten thousand parts per million) are to be measured.

2.1 The Semiconductor Gas Sensor for Reducing Agents

Insofar as semiconductor sensors for reducing agents such as CO, methane, and propane are con-

cerned, the dominant product in the market at present is the Taguchi-type sensor, manufactured by Figaro in Japan. They are widely used to detect leaks of domestic gas, to detect fires, and to detect concentrations of carbon monoxide (200 to 1000 ppm), methane, isobutane, and aromatic hydrocarbons at concentrations greater than 100 ppm. Such devices measure the change in resistance of a semi-conducting film (e.g., n-type SnO_2) at an elevated temperature (300° to 500 °C) in contact with gaseous molecular species.

The major advantages of semiconductor gas sensors are:

- Low cost
- Small size (on the order of 1 cm³)
- Ease of use (requires only a simple resistance measurement)
- Relatively high sensitivity in most cases
- Relatively fast response (typically less than 10 s)
- Long lifetimes (some units are said to have operated continuously for more than five years).

Some disadvantages of these sensors include:

- Lack of reproducibility
- Poor selectivity (e.g., detection of CH_4 and C_3H_8 is often confounded by contact with ethanol vapor; CO detection is often confounded by high cross-sensitivity to H_2)
- Humidity interference
- Long-term drift
- Nonlinear response, exhibiting an exponential dependence on concentration (usually conductivity varies as $p^{1/n}$ with $n=0.3$ to 0.5)
- Continuous power drain (usually between 0.5 to 1 W).

The most popular method of preparing a semiconductor gas sensor is the method used by Taguchi for his original gas sensors. These sensors consist of a ceramic tube with the heater element within the tube and the SnO_2 semiconductor coated on the outside. Figure 1 shows a sketch of a typical Taguchi-type sensor. Typically, the length of the ceramic tube is on the order of 3 mm; the diameter is about 1.5 mm.

Silk-screened or sputtered metal contact areas (gold) are deposited on the tube to make electrical contacts to the semiconductor coating. The coating of the tube with the semiconductor is done with the semiconductor powder in the form of a paste. Sintering of the semiconductor powder paste is a very important step in completing the sensor manufacture.

Another semiconductor gas sensor that is commercially available as a sensor for reducing agents is one produced by Panasonic based on Fe_2O_3 as the semiconductor material. It is of use for the detection of the same type of gases the SnO_2 sensor is used for.

The other area of current use of semiconductor sensors is in the detection of H_2S . Thin-film sensors of sputtered SnO_2 or WO_3 are commercially available. The introduction of sputtered thin-film semiconductor sensing materials brings us to our next topic: the application of advanced microstructure technology in the manufacture of semiconductor-based gas sensors.

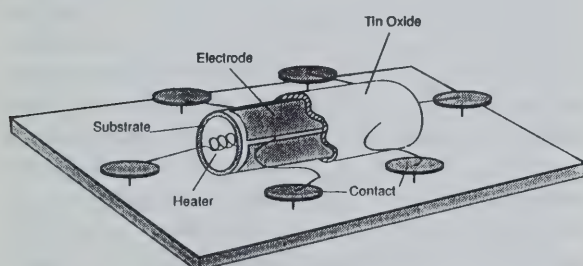


Figure 1. Structure of a Taguchi gas sensor.

To make the structure shown in figure 1 into an advanced microstructure, we must apply planar technologies as shown in figure 2. Introduction of batch, planar processes should in principle allow for more uniformity of product and lower manufacturing cost. In case we also implement a micromachined, thermally isolated heater, we might also operate the sensor with more energy efficiency. The active semiconductor, say SnO_2 , covering the heater element on the planar substrate could be in the form of a thick or a thin film. For the manufacture of a thick film, one can use silk-screening. Its major advantage over sintered or pressed powder sensors used in classical Taguchi sensors is that the use of silk screening techniques is more adaptable to mass production than most methods of preparing ceramic sensors.

Thin-film sensors can be prepared by sputtering ("reactive" sputtering—in a low-oxygen pressure) evaporation with subsequent oxidation, chemical vapor deposition, or spray pyrolysis. In the spray pyrolysis technique to provide thin films of SnO_2 one sprays—using essentially an atomizer—a metal organic form of tin or tin chloride dissolved in a suitable solvent. The solution is sprayed onto a heated substrate, and the tin compound reacts with oxygen or water vapor to form the oxide.

In the case of the vacuum techniques for deposition, a heater element is usually deposited first, then an insulator such as silica, then the tin oxide. In the case of the spray pyrolysis, where one is avoiding vacuum systems, a thick film RuO_2 heater can be deposited, although perhaps it is too expensive for some applications.

The major obstacle to more rapid progress is that the sensitivity of thin films to gases is not yet understood very well. Except for the case of the thin-film H_2S sensors, micromachined Taguchi sensors have not yet replaced their commercially available counterparts. The currently available devices, already quite small and inexpensive, will only be replaced quickly when newer technology improves upon the existing product in terms of:

- Reproducibility
- Cost
- Sensitivity
- Response time
- Concentration range
- Specificity
- Reversibility
- Stability
- Power consumption.

At this time the best course for progress in the Taguchi sensor area is to use thick-film technology for the semiconductor (better understood), use planar devices (reproducibility and cost), and concentrate on implementing less power-consuming microheater elements (power efficiency). The last point will be addressed in more detail in section 2.4, but it can already be stated here that the introduction of microtechnologies could easily bring the power requirements down by a factor of 10.

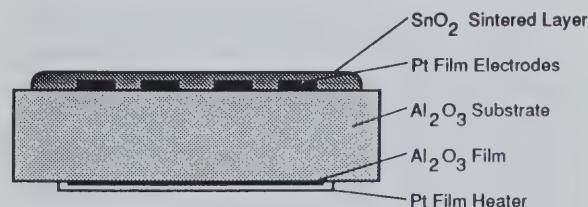


Figure 2. Sectional view of the planar Taguchi sensor.

Microfabrication cannot be pushed as a generic solution for obtaining all the above desirable characteristics. For example, poisoning of metal oxide sensors, or, for that matter, of sensors in general that depend on catalysis is a major issue that will not be resolved by implementing micromachining

technology by itself. Only the possibility of introducing redundancy could give us a way out here.

The most talked about method of improving the response of semiconductor gas sensors, especially with respect to selectivity, is by the use of arrays of sensors. This approach is by definition the ideal: by varying the catalyst, the filter, and so on, one can provide sensors with various selectivities, and by using a rather simple integrated circuit, in principle one can distinguish between as many gases as there are sensors. Unfortunately, at present the stability of the individual sensors is not yet accurate enough for this approach. We consider it impractical to calibrate such an array on the order of once a week, so drift problems make such an “array” approach impractical except for a few situations—particularly situations where only two or three gases must be distinguished. An alternate approach is that of using few sensors but changing their selectivity by operating each at a spectrum of temperatures. However, this approach has a serious problem—namely, the long time constant required to reach steady state following an abrupt change in temperature.

This is not to be negative about the future of solid-state sensors. As described above, these sensors are already commercially produced and used in magnitudes of tens of million sensors per year. Enough research is now being carried out to assure steady improvement in stability, sensitivity, and selectivity. Each improvement broadens the market as it provides the solid-state sensors with adequate characteristics, and in time these sensors will undoubtedly dominate the gas-sensing field on the basis of low cost.

2.2 The Humidity Sensor

Humidity sensing is one of the more important sensor areas, and one in which a lot of growth is expected. Significant energy savings can indeed result if dryer operations can be better controlled, for example. Attempts are now also made to apply humidity sensors to air conditioners in order to save energy.

The following are important criteria for humidity sensors:

- Good sensitivity over a wide humidity range
- Long life
- Low cost
- High stability
- Good repeatability
- Minimal hysteresis

- High selectivity for moisture
- Quick response
- Low temperature dependence.

The different humidity sensors that are commercially available can be divided into four groups:

- Hygrometers
- Psychrometers
- Dew-point sensors
- Moisture sensors.

Because I am dealing in this book with sensors rather than with instruments, I will concentrate here on hygrometers; the other classes of humidity “sensors” consist mostly of instruments. The hygrometer class consists of resistive and capacitive hygrometer sensors and mechanical displacement hygrometers. A mechanical hygrometer that also should be classified as an instrument rather than as a sensor, uses special substances (e.g., a human hair) that undergo dimensional changes resulting from changes in the moisture content. Although mechanical displacement hygrometers have been replaced for the most part by resistive or capacitive hygrometers, some industries such as paper mills and textile production are still using them. Capacitive and resistive humidity sensors differ only in the way the impedance change induced by humidity changes is monitored—i.e., the imaginary part (for capacitive) or the real part (for resistive). The capacitive hygrometer is usually based on a thin-film capacitor (e.g., a one-micron-thick hygroscopic polymer) that absorbs water molecules through a thin metal electrode and causes the capacitance to change proportional to the relative humidity.

In terms of fundamental operation principle there are two main classes of solid-state humidity sensors commercially available: one depending on electron injection into a semiconductor upon the chemisorption of water; the other depending on the ionic conductivity of the water when physisorbed on the insulator (polymer or ceramic). In the first case we are dealing with a Taguchi sensor, and the discussions of Section 2.1 do apply. In the second case we are dealing with a planar micromachined-type device.

The problems with the physisorption type of device are:

- Narrow operating range (274°–423 °K):
- Large temperature dependence
- Progressive increase in resistance when exposed to a humid atmosphere for a long time
- Conductivity errors caused by CO₂ variations.

The problems with the chemisorption type of devices (i.e., Taguchi sensors) are:

- Interference by the presence of reducing gases, especially ethanol
- High power consumption.

In general, the response for the Taguchi-type humidity sensor is stabler over a long period, and the interference effect is small at high humidity levels and high temperatures.

One of the most successful capacitive solid-state humidity sensors based on physisorption on Al_2O_3 is the Humicap sensor series produced by Vaisala Inc. It is based on Al_2O_3 . This sensor series is claimed to cover the measurement range of 0 to 100% RH within about two percent RH, to cover temperatures between -40°C and $+115^\circ\text{C}$, to remain stable over long periods, and to have fast response times (less than 5 seconds). Moreover the sensor is very small, with a negligible thermal mass. Some models are protected against fouling by a sintered filter. A Humicap sensor head costs about \$90; with instrumentation included, the price is between \$315 and \$780.

Advanced microstructure technology is already quite heavily employed in the manufacture of the Al_2O_3 based Humicap sensor, and it is clear that in the main, materials properties rather than manufacturing are limiting the wider application of these humidity sensors.

The newest developments in humidity sensors have been in the use of new materials with good reversible moisture-sensitive properties, the down-scaling of devices, and the introduction of SAW devices.

Kulwicki [1] reviews humidity sensing from a practical point of view, indicating that alumina-based sensors are nonreproducible after being exposed to a high humidity, and a $\text{MgCr}_2\text{O}_4\text{-TiO}_2$ spinel developed by Nagamoto et al. [2] is preferable, although it must be cleaned by heating to elevated temperature between readings. The proposed model with this sensor involves active Cr^{+3} adsorption sites for OH^- . Other sensors reported include $\text{ZnCr}_2\text{O}_4\text{-LiZnVO}_4$ ceramic sensors, where the Li and the Zn are introduced to vary the porosity—one wants high porosity for sensitive RH sensors [3]. Nitta et al. [4] report two multifunctional sensors, in one case using TiO_2 in a sensor to detect oxygen pressure at high temperature, and in the other case using a high dielectric sensor to measure temperature, while in both cases measur-

ing humidity as a ceramic sensor at low temperature.

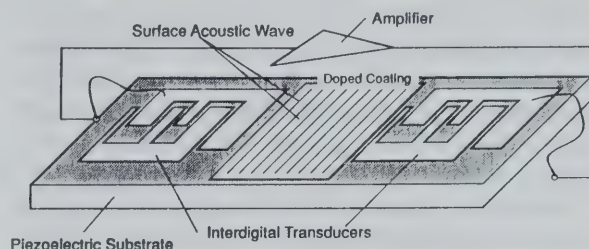


Figure 3. Coated surface acoustic wave (SAW) sensor.

Despite the possible advantages mentioned above, there has been much less work on the semiconductor-based humidity sensors, where the humidity is measured by the increase in conductivity due to “donor-like” behavior of adsorbed water on semiconductors. It was first observed with germanium [5], and since then for almost all semiconductors—that adsorption of H_2O does indeed increase the conductance for n-type and decrease the conductance for p-type material, presumably due to electron injection.

However, it is clear from a chemical point of view that H_2O is much too stable to inject electrons into either the valence or conduction band of most semiconductors. Morrison [6] suggested that the electron injection is not from the water molecule itself, but from adsorbed oxygen ions. The polar water molecule, adsorbing adjacent to the oxygen ions, changes its energy level and its rate of electron injection and extraction, resulting in a new loss in the density of adsorbed negatively charged oxygen.

SAW devices have become quite popular transducers for novel types of humidity sensors. These devices consist of a piezoelectric substrate that has interdigitated electrode arrays microfabricated at either end (fig.3). A periodic electrical signal applied to the input transducer creates a periodic mechanical deformation on the surface of the crystal. This surface wave propagates from one electrode array to the other, where the mechanical energy is converted back to an electrical signal. Because the wave is confined to a small region at the surface, anything present at that surface will cause a large change in the wave parameters over those of a clean surface. A variety of coatings can be used to make the device selective to humidity. The main advantages at this stage for these sensors are:

- Very high sensitivity
- Low power consumption.

These devices can be made for about \$100 today; eventually, though, using advanced microstructure technologies, the cost could be further reduced. In this case, piezoelectric ZnO films could be patterned on Si and interdigitated electrodes made in a batch process.

Advanced microstructure technologies applied to the semiconductor-based humidity sensors could lead to less power consumption as in the case of Taguchi sensors (see Section 2.1 and Section 2.4 on thermally isolated micro heater elements).

Despite the many humidity sensor products on the market, no good, nonfouling, fast, on-line, reliable humidity sensors are available. Despite a lot of research, few significant improvements have emerged over the last ten years. The potential market is very large—especially when household items such as air conditioners and ovens are considered; therefore, research to improve on existing humidity sensors is likely to continue. The best approach to a more reliable humidity sensor might be a SAW-based device for low-temperature and high-sensitivity applications. As indicated, this does not solve all the problems though. For high humidity and high temperature, the Taguchi semiconductive-type humidity sensors remain the cheapest solution and the most reliable for long-term application. As indicated before, we believe that the application of a microheater in a Taguchi sensor can further lead to lower power consumption.

2.3 The Solid-Electrolyte Air/Fuel Ratio Combustion Sensor

Solid-oxide-electrolyte-based sensors in conventional large shape are widely used for sensing in difficult environments today. The solid electrolytes are used as nonporous membranes separating two compartments containing chemical species at different concentrations on either side. By measuring the potential across such a membrane, one can determine the concentration of the chemical species on one side if the concentration of the species on the other side (i.e., the reference side) is known. In general, solid electrolytes allow the quantitative determination of the concentration of those species that are ionically transferred in the electrolyte (for a more detailed analysis see ref. 7). The species that one can measure with a ZrO_2 electrolyte is oxygen, O^{2-} being the species that can migrate through the zirconia lattice.

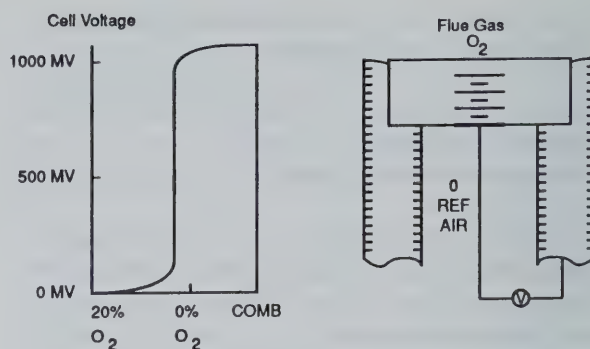


Figure 4. Voltage-mode oxygen analyzer characteristics.

Zirconia-based electrolytes, especially $\text{ZrO}_2\text{-Y}_2\text{O}_3$ (yttria stabilized zirconia, or YSZ) and $\text{ZrO}_2\text{-CaO}$ (calcium stabilized zirconia, or CSZ) have been used most extensively for a wide variety of applications:

- Thermodynamic property measurements
- Measurements of oxygen in high-temperature gases
- Measurement of dissolved oxygen in liquid metals
- Solid-electrolyte fuel cells
- Measurement of pH in aqueous solutions at elevated temperatures
- Oxygen pump to remove oxygen from stationary or streaming gas
- High-temperature kinetic studies
- Electrolytic dissociation of water into hydrogen and oxygen
- High-temperature “pseudo-reference electrode.”

Oxygen sensing with ZrO_2 for automotive use is by far the largest application. A typical automotive type oxygen sensor used today is shown in figure 4. An oxygen sensor based on ceramic ZrO_2 tubes performs well in the 600 to 1000 °C range. As temperature decreases, its performance becomes less reliable and then unacceptable. It would be desirable to identify alternative oxide-ion conductors for oxygen sensing that perform adequately at lower temperatures.

Recent developments in microionic techniques can be used to fabricate a new generation of zirconia-based oxygen sensors. These new developments are based on the possibility of preparing solid electrolytes such as ZrO_2 as thin or thick films with well-defined geometries onto appropriate substrates. The methods used are similar to those used in microelectronics: vacuum deposition, photolithography, chemical vapor deposition, silk-

screen printing, ion-beam implantation, laser annealing, and so on. An example of a ZrO_2 microionic oxygen sensor—namely an air/fuel (A/F) (also called a lambda sensor)—by Velasco and Schnell [8], is shown in figure 5. The calcium-oxide—stabilized zirconia (CSZ) layer in this sensor is deposited here by reactive sputtering. The reference electrode is made of palladium/palladium oxide and the working electrode of porous platinum; both are deposited by thick-film techniques. The voltage measured across the ZrO_2 membrane as a function of the air-to-fuel ratio exhibits an S-shaped curve with a big voltage excursion at the stoichiometric point ($\pm 1\text{V}$) (see also fig. 4). The size of the sensor shown in Figure 5 is $2\text{ mm} \times 10\text{ mm}$. This microionic sensor was shown to be more resistant to lead poisoning than the classical Lambda sensor [8]; in this planar arrangement the gases cover a much longer distance than in the case of the transverse classical ceramic structure, thus giving a better catalytic efficiency. The microionic ZrO_2 device shown in figure 5 incorporates a very thin ZrO_2 layer, and oxygen sensing at temperatures as low as 300°C is possible here because of the lower impedance associated with the thin zirconia film.

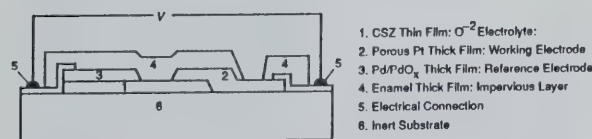


Figure 5. Stoichiometric A/F sensor—a cross section of a microionic device.

In spite of numerous tests, it has not been possible yet to find an alternative, stable oxide ion conductor operating well in the lower temperature range. A material such as CeO_2 which does exhibit a higher conductivity at the same temperature than ZrO_2 exhibits too high an electronic conductivity in reducing atmospheres.

The above described zirconia sensors were all used in a potentiometric mode—i.e., a voltage is measured across the solid electrolyte. The response characteristic in this case is logarithmic in the oxygen concentration—i.e., a Nernstian response is obtained. Nernstian response is very convenient when measuring low concentrations in oxygen (the response between 10^{-8} and 10^{-9} atm is the same as between 1 and 0.1 atm) but is not so sensitive because the voltage depends linearly on the tempera-

ture but only logarithmically on the oxygen concentration. If the concentration in oxygen is high and one wants a very accurate oxygen determination, it is better to work in an amperometric mode (fig. 6). The limiting current i in an amperometric operation mode has a linear concentration dependence and is given by eq (1):

$$i_l = 4FD(Q/L)C_o \quad (1)$$

Here D is the diffusion coefficient of the diffusing species and Q and L are two geometric quantities, namely the effective cross section Q and the effective length L of the diffusion region, C_o is the concentration of the gas species O in the gas phase.

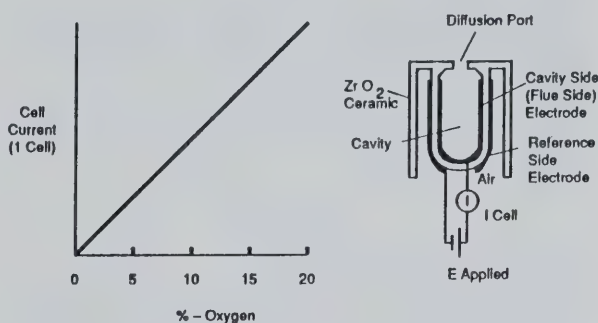


Figure 6. Diffusion-limited current-mode characteristics.

There may be a problem in applying this formula to solid electrolytes. The formula was derived for aqueous systems, and there is a question about whether limiting current conditions can also be realized in solid-state cells with gas diffusion. Limiting currents can only be measured when the rate of the reaction products at the electrode, and hence the current flowing, is determined exclusively by diffusion. In an amperometric (also called polarographic) sensor, the activity of the electrode must be so great that all the oxygen diffusing in can react with ease, so that the concentration at the electrode is zero. The diffusion in a gas proceeds much faster than in a solution, so that the resulting diffusion currents are in general so high that the electrode is incapable of reacting all the gas diffusing in (for example, D for O_2 in water at 25°C is $2.4 \cdot 10^{-5} \text{ cm}^2\text{s}^{-1}$ and D for O_2 in N_2 at 20°C is $0.16 \text{ cm}^2\text{s}^{-1}$). To counter this problem and force a diffusion regime, a diffusion barrier has to be created. Dietz [9] made such a sensor by applying a porous ceramic coating over the amperometric device with an effective pore cross section diameter low

enough to provide resistance to O_2 passage—enough resistance that a diffusion regime is created but not so much resistance that the limiting current becomes too low. During operation of such a sensor a voltage sufficient to pump oxygen in through the cathode and back out through the anode is applied across the membrane, and the limiting current is linearly dependent on the oxygen concentration; the amperometric oxygen sensor by Dietz is not really a microionic device because the solid electrolyte is a self-supporting 1-mm-thick ZrO_2 membrane, but electrodes and the diffusion barrier were made by screen printing and, of course, the principle of operation described here is general and covers classical bulk sensors as well as microionic devices.

The progress in making microionic sensors commercially available has been very slow. The materials problems to overcome in their construction are still tremendous. Still, there has been steady progress, and we can look forward to seeing the first microionic ZrO_2 sensors by early 1995.

2.4 The Catalytic Bead Sensor

Catalytic bead sensors or hot-wire catalytic sensors are in wider use than the Taguchi sensor discussed in section 2.1. They are most commonly employed to detect methane and other combustible gases. The combustible gas sensor is made up of two elements: an active or catalytic element and an inactive or reference element, both of which are exposed to the test atmosphere. Any oxidizable (combustible) gas that encounters the hot catalytic bead reacts rapidly, liberating heat in proportion to the heat combustion for the particular reacting chemical. The resistance of the platinum coil in the center of the sensor changes with temperature; this change is used as a measure of the gas concentration. The sensing element in such devices is thus basically a platinum resistance thermometer, consisting of a platinum coil heated to 500 to 900 °C. Catalytic devices of this kind are generally sensitive to combustible-gas concentrations in air between approximately 0.1 percent (1000 ppm) and 5 percent (50,000 ppm).

The major advantages of hot-wire catalytic sensors are:

- High sensitivity to methane and good sensitivity for all types of town gases
- Fast response time (typically a few seconds)
- Small size, mechanical ruggedness.

Disadvantages of these sensors are:

- No selectivity: almost undifferentiated response to hydrocarbons, alcohols, CO, and H_2
- Nonlinear response to CH_4 at concentrations between 5 and 10 percent; diminishing sensitivity at CH_4 concentrations greater than 10 percent
- High power consumption (up to 1.5 W)
- Response affected by absence of O_2 .

In the current technology, a high power on the order of 1.5 W is continuously supplied through the Pt wire. As we pointed out in the case of the Taguchi sensor, it is obvious that it would be desirable to develop a planar microcatalytic sensor that has a minimal thermal mass so as to minimize the power consumption of the device. Until this film-catalysis is better understood, it is probably better as in the case of the Taguchi sensor to continue to use thick-film technology for the catalyst.

Silicon micromachining is an ideal tool to fabricate a thin silicon membrane on which a multilayer structure with a minimal thermal mass can be built. As in all the other cases discussed above, the application of a batch fabrication process approach, typical for silicon-based devices, to the manufacture of catalytic sensors will dramatically reduce the fabrication cost, while maintaining the advantages of a catalytic bead sensor. There are several research efforts under way now in the U.S., Japan, and Europe to microfabricate the best possible heater.

In figure 7, an example of such a planar microcatalytic gas sensor is shown. Such a proposed planar-type microcatalytic gas sensor has several advantages:

- Low power consumption (estimated at less than 100 mW to operate at 600 °C)
- Possible integration of sensing and reference element
- Possible fast response time through small time constant
- Small size and light weight.

A miniature device has lower power requirements to achieve the operation temperature of, say, 600 °C. At these temperatures, radiation heat transfer dominates over natural convection and conduction through a heating element. Therefore, as the size is decreased, the radiation component decreases with the surface area. We estimate the power necessary to operate the proposed microsensor will be less than 100 mW—i.e., one-tenth of that used in the existing catalytic bead sensor. We

believe that the first micromachined products in the catalytic bead area and Taguchi sensor area will appear around 1993.

3. Electrochemical-Based Sensors

The main reason why the development of silicon-based chemical microsensors has been slower than that of, say, silicon-based pressure sensors is that in a chemical sensor the direct contact between the sensor and the fluids and gases to be analyzed poses more severe technological problems for the integration of the chemically sensitive area with the electronic functions of the sensor. These technological problems are:

- Protection (encapsulation) of the electronic functions from the often corrosive environment
- Light sensitivity of the electronic functions (e.g., the gate of the FET)
- Instability of electronic materials exposed to a gas or liquid environment (e.g., the hydrolysis of gate dielectrics)
- Lack of reliable microreference electrodes (for ion sensors)
- Instability problems due to contamination
- Poor selectivity
- Short lifetime in general
- Unavailability of multispecies sensors
- The lack of a mass fabrication methods (e.g., because of the lack of compatibility between depositing different ion-selective membranes and IC technology).

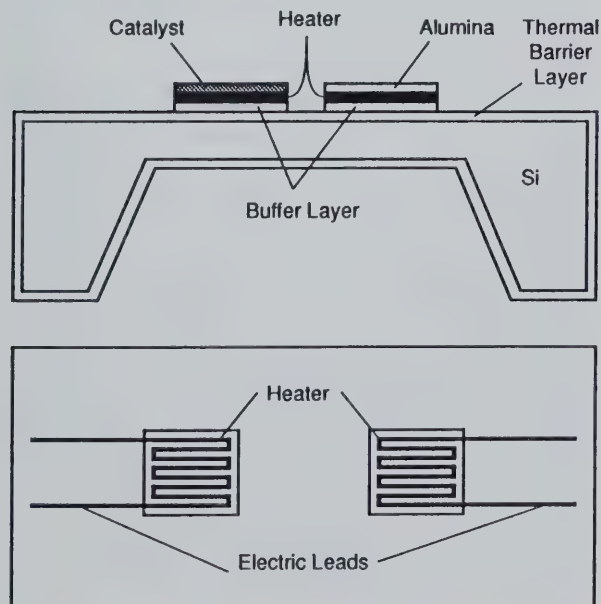


Figure 7. A planar microcatalytic sensor.

A direct consequence of the above problems is that the ability to achieve closer integration of electronics and chemical or biological sensing functions is much less straightforward than in the case of physical microsensors. The cost-effectiveness of the integration is much less clear.

It is our opinion that the research in the area of microchemical sensors has until recently been somewhat misguided. The need for developing better ion-selective membranes (better in terms of long-term stability, reversibility, response time, and other parameters) and methods of depositing these membranes, which are the real critical areas, was obscured by the early enthusiasm generated by the invention of the ion-sensitive field effect transistor (ISFET). The miniaturization and the chemistry improvements both were attempted at the same time, possibly preventing breakthroughs in either area. Also, many alternatives for potentiometric microchemical sensors that were suggested in the wake of the invention of the ISFET were not explored far enough. In many instances these alternative chemical microsensors are better approaches than the original ISFET design.

The problems with micro-gas sensors are in principal less severe than those of ion sensors. The referencing is indeed simpler, and leakage is no problem, so encapsulation is also less difficult. Total integration is usually not the best strategy in the case of a gas sensor. Because silicon electronics are limited to temperatures around 150 °C, when integrating Si electronics with the gas-sensitive material one cannot operate at the high temperatures that lead to fast response and good selectivity to gases. Moreover, many industries require sensors that can withstand substantially higher temperatures than 150 °C. Despite the fact that there is a lot of research on integrated gas sensors, the more popular commercially available gas sensors are still classical Taguchi-type sensors with the electronics completely separated from the sensing elements (see fig. 1). The gas-sensing elements in the Taguchi sensor are small already, so a closer integration of the electronics with the gas-sensitive material does not lead to significant advantages. To the contrary, integration would create unnecessary complications in this case. In the area of gas sensing the breakthroughs are expected to come from "smart" arrays of electrochemical or solid gas sensors, cheap flat planar production processes, small heater elements consuming less power, and the development of new low-temperature solid electrolytes.

In the subsection below we first discuss totally integrated (on silicon) devices (section 3.1 and 3.2) and then devices where the electronics are somewhat separated from the sensing function (section 3.3 and 3.4). Commercial devices are available in each category, and for each category it is clear that as the device performance improves, the market will expand.

3.1 ISFET

As mentioned above, in the case of the ISFET the encapsulation is a big problem because of the close proximity of liquids and the electronic functions. With the resultant compromises (e.g., a short lifetime for the sensor), the question whether integration of ion sensors is practical from an economic standpoint still remains. But irrespective of the answer to that question and other unresolved issues, discussed below, major advances have been made in the last 15 years in the understanding of various sensing mechanisms and sensor designs.

Another equally important problem related to the practicality of the ISFET is the one of the reference electrode. A conventional reference electrode, such as a saturated KCl solution with a Ag/AgCl electrode and a liquid junction, contacting the solution to be analyzed, often has been used for fixing a solution potential. Since the reference electrode is not in the same plane of the ISFET, a planar technology for fabricating the sensor cannot be used, and the cost of making such a sensor is prohibitive (for example there is manual labor involved in positioning the reference electrode). It is also difficult to achieve a reference electrode on the same scale customary in Si integrated-circuit technology, and troublesome maintenance is required as well. More recent efforts concentrate on making solid-state reference electrodes in which two ISFETs are combined: one sensitive and the other insensitive to the species of interest. For example, parylene and Teflon have been used as the insensitive electrodes. In this case, a flat planar technology is possible, but not having a "true" reference electrode for some applications still is a setback. There are also some serious scientific doubts that the parylene gate idea is workable. Using Ag/AgCl as a pseudo reference electrode is the most attractive alternative at present. But also this approach has its problems when used in a flow system due to the finite solubility of AgCl.

Also, many of the problems mentioned before—such as the light sensitivity of the gate, the encapsulation, instability of gate materials contamination, poor selectivity, lack of a mass fabrication technique, as well as lack of calibration stability (caused, for example, by photo-induced junction currents and thermal sensitivity) and poor membrane adhesion—remain.

It is a serious question whether a CHEMFET, aside from a simple pH ISFET, can be made cheaply enough. The true advantage of the ISFET technology has always been said to be the prospect of using a multiple ion sensor on a single microprobe, for example in a catheter. The technological difficulties associated with the marriage of ion-selective membrane materials and methods with silicon planar device processing are obviously postponing the realization of such arrays. One of the difficulties mentioned earlier lies in the diversity of membrane materials and the resultant variation in deposition techniques (sputtering, CVD, solution casting, evaporation, etc.). The real challenge of microchemical sensors, we believe, is to make reproducible membranes with microfabrication techniques and to fix them to a microsensor—all at a cost that the market can bear. The electronic vehicle used is in most cases of secondary importance.

Despite the problems described above, ISFET products have been introduced to the market, or have been announced, by companies such as Cordis, NEC, Thorn EMI, CHEMFET, and Kuraray. However, as said before, we believe that at the time of writing this chapter the cost-effectiveness of these devices for a big market is still in doubt. Most of the introduced products have actually failed commercially so far.

At present, the pH-sensitive, inorganic-gate ISFET is the only type that can be manufactured without major problems. This is the case after more than 20 years of intense research. All the modified ISFETs are still largely in the experimental stage. The inorganic gate materials in use for the pH-sensitive ISFET (e.g., with Al_2O_3) can be made relatively cheap, and the technology is compatible with standard IC technology. Also, the drift associated with these simple gate materials is often predictable and can be compensated for by an on-board ROM chip. On the other hand, even this simple product has not been successful yet.



Figure 8. The CHEMPRO 1000 sensor system.

ISFETs that are sensitive to other ions are all still subject to limited selectivity, short lifetime, and poor technological performance, and it is also difficult to see how one could compensate for the unpredictable drift in more complex membranes such as the ones used in, let us say, K^+ sensing (e.g., valinomycin in a PVC membrane with plasticizer). It is our opinion that ISFETs today are presumably a good idea for single-use applications in the biomedical field, but for most other analytical or industrial on-line monitoring applications, other solutions are to be opted for. The ISFET pH sensor is indeed very useful (better sensitivity, faster response time, smaller, etc.) for *in vivo* short tests, but it is inferior to the glass electrode in many respects when long-term stability is required. The latter is most likely the explanation why companies—such as Beckmann, Corning, and Orion—that are producing classical ion-selective electrodes (ISE), are not initiating products in this area (the classical ISE producers seem to have opted for solid-state thin-film ISE electrodes instead).

Multisensor ISFETs will presumably have their main application in the biomedical field because cost is less important, and the device does not have to last too long. These multi-ISFETs will reduce the analysis time substantially; they also will give greater sensitivity as well as exhibit faster response times than classical ion-selective electrodes. Sibal [10], for example, showed how a multisensor ISFET mounted in a small block and attached to the arm of a patient could simultaneously measure H^+ , Na^+ , K^+ , and Ca^{2+} ions. A fully automatic system takes blood samples, flushes the ISFET cham-

ber, and attaches tubes with calibration liquid during a fixed cycle, while the microprocessor controlling this cycle calculates the concentrations of the ions. Only 0.6 ml of blood is used per sample. We believe this application “out of the body” can be more easily and less expensively accomplished today by using more classical technology (see, for example, the Sentech sensor in fig. 8). On the other hand, a sensor for blood gases and ions mounted in a catheter would warrant the cost of going completely integrated.

Summarizing, ISFETs are presumably not the best solution for most continuous on-line chemical monitoring problems. For biomedical applications and as throwaway devices, they do have a chance, especially with gate materials that are easy to make reproducibly. For multi-ion sensing, the cost effectiveness of this approach is still a big question, and better, simpler solutions might be available.

3.2 Gas-MOSFET

Reflecting the somewhat less complex technological problems to overcome for an integrated gas sensor compared to an integrated ion sensor, integrated gas sensors came on the market very quickly after they were conceived. They have not been large commercial successes up to now.

An example of a totally integrated gas sensor is the Pd-gated MOSFET invented by Lundstrom [11] for the detection of hydrogen and shown schematically in figure 9. Sensor in Sweden [12] and Tricomp Sensors Inc. (not in existence any longer) in the USA have brought H_2 sensitive Pd-MOSFET sensors on the market. Sensor has improved its product recently, for example by implementing an Al_2O_3 gate insulator, dramatically reducing the hydrogen-induced drift [13]. Intensive work to explain the theoretical basis of this type of sensor was done by several authors. There is a lot of controversy still lingering; for example, some authors have shown effects due to hydrocarbons at room temperature; other authors speculate that these results are to be explained by hydrogen impurities in the gas.

With gas MOSFETs where holes are present in the metal gates, CO was detected at relatively low temperatures, about $150^\circ C$ [14]. In these devices the chemically sensitive material is deposited immediately on top of the amplification function. There are some major problems with devices based on this principle (apart from zero point drifts typical for MOS devices). First of all, at relatively low temperature, the influence of humidity is expected

to dominate and to swamp out any specific reaction with the gas of interest. Chemisorption processes will be dominant only at relatively high temperature, and for obtaining selectivity chemisorption is preferred over physisorption. As we have seen before, the temperatures one can cover with Si are not high enough to get into that operation mode, and integration of gas sensors is again not so advisable. Also, for the more inert gases the response time will be slow, and aging problems due to mobile charge in the dielectric will be more severe at somewhat elevated temperatures. Poisoning of the metal electrodes is quite likely, especially in the presence of sulfides.

The lower detection limit is also a sensitive function of the ambient gas. Although there have been very encouraging reports lately on gas MOSFETs, we suspect their application range will stay rather limited in the near future. We do not see significant advantages in trying to integrate the sensor so closely with the electronics. In the case of some room-temperature solid electrolytes and organic materials, the situation might be more favorable for total integration.

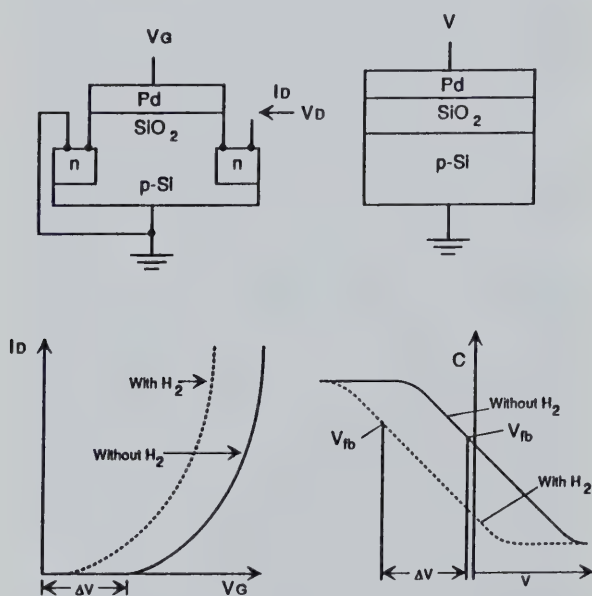


Figure 9. Hydrogen-sensitive MOS structures.

3.3 Hybrid Devices and Miniature ISEs

In hybrid structures, the electronics are kept separate from the chemically sensitive area, but both are on the same substrate in close proximity to each other. In this class, thick- and thin-film sensors are distinguished.

There are very few reported hybrid-ion electrodes. An early example of a thick-film hybrid sensor is shown in figure 10. In this case, the pH-sensitive membrane is connected with a short metal wire to the electronic chip on the same ceramic substrate. The electronic chip can be bought cheaply and is usually already encapsulated. Of course the encapsulation makes the manufacturing of such a device a lot simpler, and if the signal-line length is kept short enough, a lot of the advantages of the more integrated devices still remain. Although the devices will be necessarily larger than integrated devices, multiple ion sensing is still possible.

Some of the work closest to a practical hybrid ion sensor has been done by Fjeldly et al. [15], an example of which is shown in figure 11. In this case an op-amp chip on a ceramic substrate was connected to a membrane. By the strict definition of the word *hybrid* this is not really a hybrid sensor because the membrane is not mounted on the same ceramic substrate as the electronic chip with the electrical connections. In the case of an LaF_3 membrane, Fjeldly uses as a reference electrode a solid-state reversible contact (Ag/AgF) on the back of the membrane. This reference electrode can be compared to the liquid fillings used in the classical F- electrode. This, by itself, represents a considerable simplification. Such a solid-state reversible contact to a solid-state ion-selective membrane also leads to dramatic improvements in performance (e.g., the long-term drift is reduced) over the equivalent capacitive devices in which the ion-selective membrane is placed directly on the gate of a FET. This move away from capacitive devices is now popular in the scientific community. One tries to implement symmetrically bathed membranes instead of relying on the blocked interface typical for the ISFETs.

The hybrid approach seems the best to date for the sensing of a small number of different ions (for example, of Cl^- , pH, and Na^+ in blood), especially when considering applications outside a living body, where size is not particularly important but cost is. For chemical sensors where the lifetime usually will be low, it also seems better not to try to make each sensor "smart" but to leave intelligence in a separate custom-type microcomputer. Along this line we can point to the earlier discussed ChemProTM 1000 system from Sentech shown in figure 8. Each of the disposable ChemPro cards shown (about \$4) is a precalibrated, solid-state sensing electrode system that provides from one to four different tests (e.g., pH, Cl^- , Na^+ and K^+)

in combinations appropriate for varying health care requirements. Each of these one-time-use ChemPro cards incorporates sensors, which are actually miniature ion-selective electrodes, that are individually fabricated into the molded card. All electronics and software are in the analyzer instrument (about \$7000). Again, this sensor is not a real hybrid sensor, because no electronic functions are incorporated into the card, but this concept of miniaturization and multiple functionality is very timely. If the concept permits closer integration of the chemistry with some op-amps it becomes necessary—e.g., if, with the introduction of more sensing, function crosstalk becomes too difficult to handle with the present long signal lines. The experience gained in manufacturing the ChemPro Cards should be good preparation for this next step.

It is surprising to see that so few new products have been reported in the area of hybrid chemical ion sensors. It seems that a multiple-purpose hybrid-ion sensor for, let us say, the automatic tuning of swimming pool chemistry, or the control of fermentor chemistry would be commercial successes. As indicated above, the difficulties to overcome are much less severe in the case of the hybrid structures than in the case of more closely integrated structures. Thus, at low production rates, the hybrid devices could be made at less cost than, for example, ISFETs. If big quantities eventually are required one might then consider a more closely integrated sensor, in which case some type of an EGFET approach may be desirable.

The case of hybrid sensors for gases is different again. In the case of gas MOSFETs, we mentioned that for a satisfying operation a high temperature is required, and that puts one outside the range of what is possible with a closely integrated Si chip. Again, for hybrid sensors the distance between the electronics and the gas-sensitive elements has to be large enough to allow temperatures of up to 400 °C at the sensing element.

3.4 EGFET

The extended gate field effect transistor (Lauks and Van der Spiegel, Integrated Ionics and University of Pennsylvania) [16] is, in a sense, intermediate between the totally integrated sensor and the hybrid sensor. It consists of an integrated guarded coaxial line, connected to a high-input impedance on-chip preamplifier (see fig. 12). The chemically sensitive membrane is deposited on the signal line and generates a signal that is transferred over the

coaxial line to the preamplifier. The chip “floor plan” is in essence separated into two sections, with the electronics separated from the ionics. This approach reduces packaging, shielding, and crosstalk problems to a large extent. Integrated Ionics has almost finished development work on an eight-channel, multivariate chemical microsensor with on-chip low-power CMOS integrated circuitry. The deposition of the different membranes was done entirely at the wafer level using planar photolithographic techniques. Along the lines of the Fjeldly work described above, I-STAT has decided that rather than making completely blocked electrodes, solid-state reversible contacts for solid-state ion-selective electrodes and symmetrically bathed configurations for polymeric liquid-type membranes will be used for improving drift problems. If the cost of manufacturing is manageable, this sensor approach should be superior to the ChemProTM card.

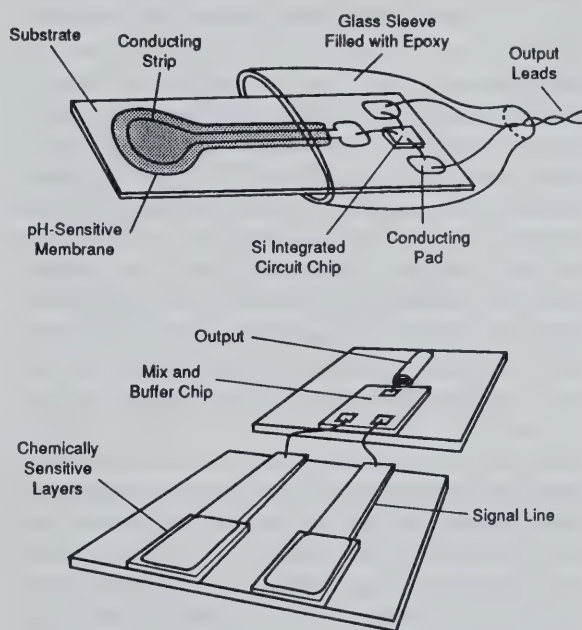


Figure 10. Hybrid device structures.

4. Markets for Chemical Sensors

4.1 Current Sensor Applications

Four major markets currently exist for chemical sensors: the toxic and combustible gas sensor market, the automotive market, the energy-conservation (e.g., all drying operations) market, and the medical market. Each of these markets accounts for over \$100 million in sales per year in the United

States (especially in the medical field the market is much larger), including the sensors and the electronic systems such as signal processors, displays, and alarms to which they are attached. In most cases, a single supplier sells the electronics and the sensors as a package to end users.

4.1.1 Toxic and Combustible Gas Monitoring

The toxic and combustible gas market is the most established market for chemical sensors. In the U.S. the largest current use of gas sensors is in environmental monitoring and control applications in industry rather than in the home (in Japan the home market is at least as big). More specifically in the U.S. chemical sensors are used primarily in the petroleum, petrochemical, mining, and semiconductor industries. In the first three industries, the most common gases monitored include hydrogen sulfide, carbon monoxide, chlorine, methane, and flammable hydrocarbons. The most important gases monitored in the semiconductor industry are phosphate, arsine, and silane. The prevalence of hazardous and combustible gases in the petroleum industry is so high and the danger to workers and equipment so great that the impetus to develop chemical sensors for monitoring their presence is strong. Government regulations have further stimulated monitoring requirements. Until about ten years ago, the sensors made for this purpose were not very accurate, specific, sensitive, or long-lived, but due to their great need the users put up with their shortcomings. More recently, chemical-sensor technologies, particularly electrochemical and semiconductor-sensor technologies, have improved substantially, resulting in a number of new products.

For the past few years, however, the market for toxic and combustible gas sensors has been stagnant because of the collapse in oil prices. Currently, the market for small solid gas sensors and sensor systems is some \$120 million in the United States and in excess of \$230 million worldwide. Broken down by product, the U.S. market for these chemical sensors comprises about \$25 million for hydrogen sulfide monitoring equipment, \$20 million for total hydrocarbon monitoring equipment, \$12 million each for carbon monoxide and oxygen deficiency monitoring systems, and small percentages for systems to monitor myriad other gases. When the oil business recovers, this market is likely to return to its previous 10% annual growth rate. New business developments in this area will probably wait until advances in sensor fabrication technologies lower unit costs to the point that home air monitoring is economically feasible.

Nonindustrial applications are only beginning to emerge now—e.g., gas sensors for places where bottled gas is used (RVs, boats, mobile homes, etc.). The aspect of home safety, climate control in buildings, and smart kitchens seems to be pursued much more energetically in Japan and Europe than in the U.S.

4.1.2 Automobile Combustion Control The automotive market has been another major development area for chemical sensors. Demand for reliable sensors in this business is quite high, and the large market justifies the high development costs for the sensors and the complex fabrication processes required to reduce unit costs. Once developed to the technical and economic specifications of the automakers, these sensors find wider use in industrial applications that, by themselves, would not justify high development costs. Similar market evolutions in the chemical sensor business will occur many times in the coming years.

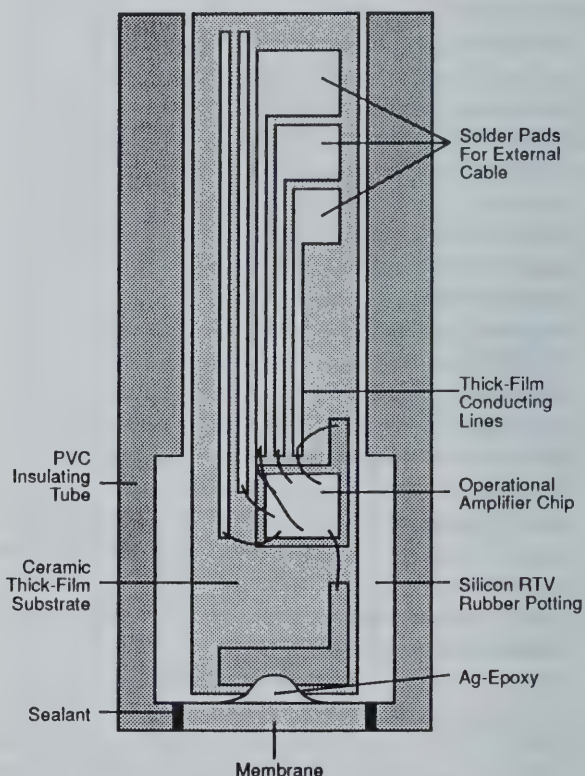


Figure 11. Details of the ion-selective electrode.

The chemical sensor of greatest interest to automakers is the oxygen sensor, which is used to optimize the combustion process in the engine. The value of precise combustion control rose significantly after the price of gasoline increased in 1974

and the U.S. government began to regulate fuel efficiency in cars. As a result, a cheap oxygen sensor made from zirconia oxide was most developed. Such sensors—commonly known as lambda sensors—are used in most automobiles sold today. They are extremely durable, long-lived, and quite accurate and sensitive. Their major drawback is that they do not operate below about 300 °C, and therefore cannot be used for the many possible room-temperature applications of oxygen sensors. Future automotive chemical sensors will be exhaust gas monitors for pollutants and humidity sensors for climate control of the interior of the vehicle.

The market size for automotive oxygen sensors is difficult to assess because most of them are produced captively by automobile manufacturers. Oxygen sensors (including some electronics) cost about \$20 each; the total U.S. market for automobile oxygen sensors alone is probably about \$100 million to \$120 million.

4.1.3 Energy and Drying-Operation Markets

Humidity sensors can make drying processes much more efficient, and the energy savings generated by humidity sensors could be very large. Indeed, drying was estimated to consume from 1.2 to 1.8 quads (1 quad = 10^{15} BTU) of energy per year in the U.S. in 1980 (about 6% of the total energy use in that year). Major industrial groups where reliable humidity sensors are needed are listed in Table 1. The market for humidity sensing in the U.S. is about the same size as or larger than the total toxic gas sensor market (\$120 million).

4.1.4 Medical Analysis The medical market for chemical sensors is most promising and has attracted a great amount of interest among researchers and business planners and developers. The potential applications for chemical sensors in the medical market are extremely diverse—much more so than for any other market. The cost constraints in the medical market are not nearly as tight as they are in the industrial market, and the potential market size for a host of different sensors is very large. Both factors amply justify high devel-

opment costs. Perhaps the most important characteristic of the medical market from a technical standpoint is that many of the applications call for disposable sensors. One of the most difficult features to achieve with chemical sensors—stable response over a long period—is not necessary in the medical market. This factor simplifies sensor development considerably. A major problem in this area, however, is often blood and/or tissue compatibility. Funding sources are becoming critical of any sensor approach whose developers do not address blood and/or tissue compatibility problems carefully in the case of in vivo sensors. Because of the latter, many companies developing biosensors have chosen to address ex vivo sensing first.

The largest applications for chemical sensors (and biosensors) in medicine are for blood and urine chemical analyses. The largest sensor market will be for clinical laboratory testing equipment for individual samples for blood gases (oxygen, carbon dioxide), and pH, glucose, sodium, potassium, calcium, chloride, bicarbonates, urea, creatinin, bilirubin, and cholesterol.

Systems for this market today are generally benchtop analytical devices; revenues from sales of systems and supplies for this segment are approaching \$1.2 billion per year in the United States. Strong growth is expected in this market, with a major driving force being new and improved sensors that lower assay and equipment maintenance costs and shorten assay times. As dependable, disposable sensors appear, much of the blood testing presently done in labs will be done directly in physicians' offices or, in some cases, in patients' homes.

In addition to the established laboratory equipment market, another market is emerging for continuous monitoring devices to analyze patients' blood chemistry. Of particular importance are chemical sensors for blood gases, glucose, and blood electrolytes. However, continuous blood-gas analyzing equipment accounts for most of the business at present. Sales of continuous blood-gas monitoring equipment were about \$115 million to \$150 million worldwide in 1986. Four sectors characterize this business: devices that measure oxygen saturation levels in the blood via color measurements through the skin (worldwide sales of \$50 million to \$65 million), transcutaneous devices that measure oxygen and carbon dioxide that diffuse through the skin (\$20 million to \$25 million), devices that measure inspired and expired gas concentrations (\$30 million to \$40 million), and devices that measure

Table 1. Major Industrial Groups with Strong Needs for Humidity Sensors

- Agriculture (e.g., humidity sensor for grain elevators)
- Mining (e.g., drying of ores)
- Food (e.g., drying of fruit, microwave humidity sensor, etc.)
- Textile
- Lumber and wood
- Chemical and allied products
- Residential use, clothes dryers, dishwashers, etc.

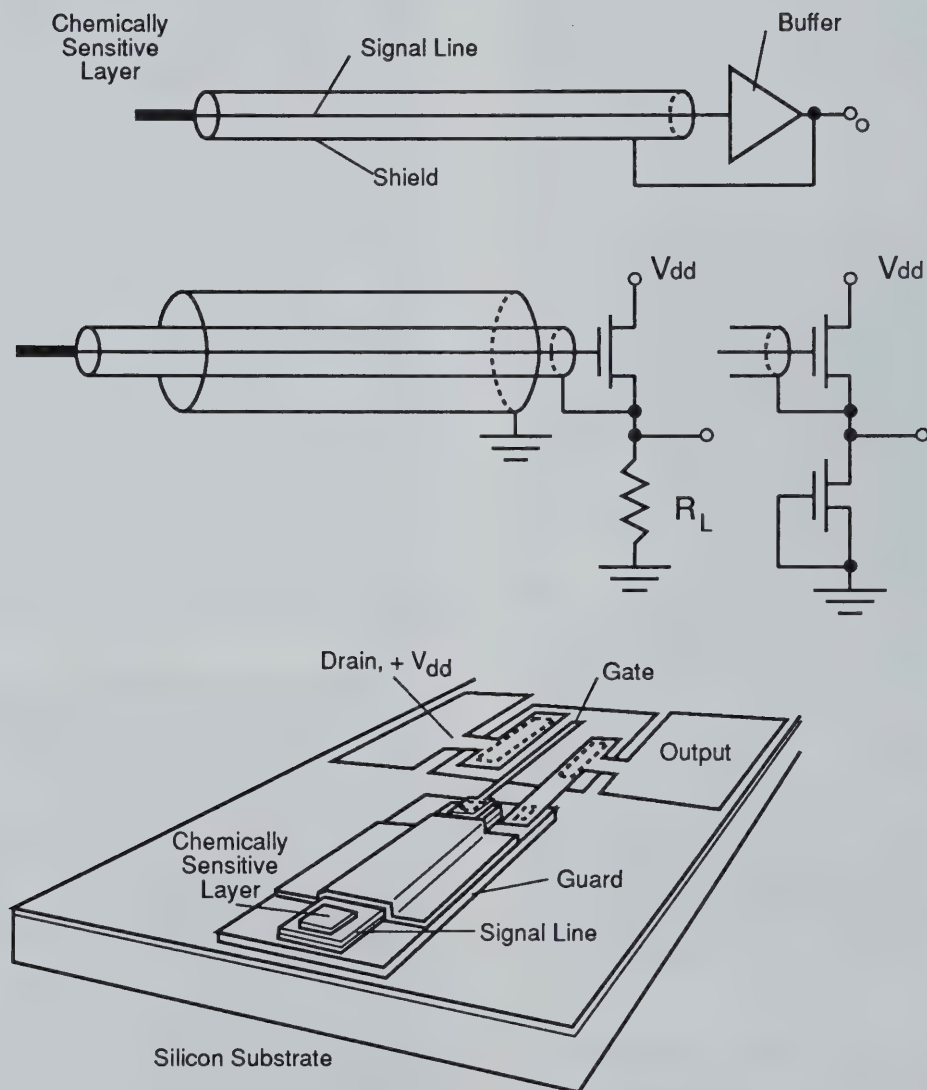


Figure 12. Extended-crate ISFET structure.

blood gas levels directly in the blood that passes through extracorporeal shunts (\$15 million to \$20 million). The market for sensor devices that monitor patients continuously is likely to grow rapidly as sensor technology breakthroughs occur and as potential users become aware of the improvements the new technology offers in patient health care.

Each of the four current markets for chemical sensors has special characteristics that negate some of the fundamental limitations of chemical sensor technology in its present form. The toxic and combustible gas market involves measuring very common dangerous industrial gases. Even inaccurate, unstable, and expensive sensors are better than none. The same holds for the humidity sensors market. In the automotive market, the need for improved combustion and pollution control was so great for so many automobiles that economies of scale made high sensor development costs acceptable. In the medical market, health-care requirements translate into relaxed cost requirements and acceptance of sensor disposability. With lower cost and longevity requirements, many chemical sensor developments are more economically feasible. When chemical sensor technologies advance beyond their present state, many other market niches will become more attractive.

4.2 Emerging Sensor Applications

Because of their technical limitations, chemical sensors are only beginning to penetrate the many potential markets in which they could be very valuable products (see fig. 13). This section identifies some of the important application areas that will emerge as sensor technologies improve in the coming years. Major market penetration of these areas is estimated at being five years or more away.

4.2.1 Defense Some years ago, nerve gas sensors were one of the most important U.S. Department of Defense (DoD) applications. The DoD funded several large projects to develop a portable mass spectrometer for accurate low-level nerve-gas monitoring on the battlefield. A natural addition to this device would be a chemical sensor capable of sensing nerve gas in small quantities. What the chemical sensor would lack in absolute accuracy, it would make up for in size, portability, and ease of use. It is possible to design a badge-sized monitor that soldiers could easily carry around. Some research groups are trying to develop such monitors using surface acoustic-wave devices, ChemFETs, or Langmuir-Blodgett film technology.

With the big government cutbacks, less progress is expected in this general area unless the FAA makes bigger efforts in funding programs for airport security against explosives.

4.2.2 Consumer Products The potential consumer applications of chemical sensors are very broad. Because the consumer market demands extremely low costs, however, few consumer applications are likely to become a reality in the next five years. An interesting application of chemical sensors in the home is a carbon monoxide sensor used as an improved early-warning smoke detector. Current smoke detectors respond slowly and nonselectively, but they cost only \$10 to \$20. The technology now available is based on ionization radiation or optical particle detection. An automatic swimming pool chemistry sensor would be another attractive consumer product. Such a sensor system would combine pH, chlorine, and hydrocarbon or ozone sensors that would control the injection of antimicrobial chemicals into the pool. Other applications include ethanol sensors in microwave ovens to improve cooking quality, combustion control sensors in furnaces and water heaters (oxygen, nitrous oxide, and methane sensors) to improve combustion efficiency, air-quality sensors in the home to detect natural gas leaks and poor ventilation, turbidity sensors in washing machines, and humidity sensors in clothes dryers. The "smart home" concept, which incorporates many of these gas sensors, calls for central control of the temperature, humidity, and ventilation of the home as well as central monitoring of air quality. The goal is tighter, better insulated homes that use less energy for heating. Such homes would need intelligent monitoring of ventilation to prevent the air from becoming stagnant or being poisoned by furnace gases or gases released by cooking food.

4.2.3 Automobiles Emerging automotive applications of chemical sensors include humidity sensors attached to the climate-control systems of cars, sensors to determine when a car's oil needs to be changed, and sensors to detect exhaust pollution. The last category includes sensors for nitrous oxides, sulfur oxides, and carbon monoxide. As pollution-control laws tighten, monitoring and controlling combustion products will require increasingly sophisticated sensors.

Some possible chemical sensor applications for the home and the car are summarized in table 2.

4.2.4 Biotechnology Applications of chemical sensors in the growing biotechnology industry consist primarily of fermentation to control devices to

Table 2. Some Micro Chemical Sensor Applications for the Home and for Cars

Area of Application	Humidity	Gas	Ions	Other Chemical Information (e.g., turbidity, dielectric constant, biochemical, etc.)
small boilers		+ (O ₂)	+ (water hardness)	
safety in garage, home, etc.		+ (CO, CH ₄)		
washing machine				+ (turbidity with e.g., light scattering)
microwave oven	+	+ (ethanol)		
food freshness	+			+ biochemical degradation products
clothes dryer	+			
room air conditioner	+	+ (refrigerand)		
dehumidifier	+			
exhaust fans	+	+ (CO)		
dishwasher			+ (water hardness)	
pool			+ (pH, Cl ⁻ , etc.)	
car:				
air cond.	+			
combustion		+ (O ₂ , CO, SO _x , NO _x)		
oil change sensor				+ (e.g., dielectric constant)
rain sensor				+ (e.g., transparency of the window)
soot sensor				+ (e.g., dielectric constant)
for diesel				
etc.				

lower cost by improving product yields and increasing process uniformity. Examples of such sensors include dissolved oxygen and carbon dioxide sensors; sodium, potassium, chloride, and calcium sensors; pH sensors; and sensors for various sugars and metal ions. These devices are the more generic sensors that could indirectly indicate process yields and directly measure fermentation progress. Eventually, sensors may be able to measure directly the complex molecular species being formed in the fermentation vats. Combinations of these sensors will provide the information necessary to optimize and control the fermentation process.

Sensor systems of this type will also find wide use in the pharmaceutical industry. The need for sterility is one of the major problems with these applications. If the sensors are to make in situ measurements, they must be able to withstand the steam sterilization process used to clean the vats between batches. Many sensors designed to sense biological species contain active enzymes that decompose at temperatures above body temperature. Thus, the mechanisms on which these sensors depend cannot coexist with the standard sterilization processes.

4.2.5 Wastewater Treatment Wastewater pollution is an increasingly important problem for a

host of industries, including pulp and paper, semiconductors, electroplating, chemicals, mining, and nuclear power. Monitoring and controlling the waste levels in discharged process water will be yet another problem that chemical sensors can help solve. Wastewater treatment is expected to be one of the greatest opportunities for chemical sensors. Among the most important chemical species to be monitored will be hydrocarbons in general and process solvents in particular, as well as heavy metal ions, dissolved ammonia, chlorine, and pesticides.

4.2.6 Combustion Control The drive to improve combustion efficiency and reduce pollutant gas products is very strong. The use of control systems based on oxygen intake and consumption in combustion processes is slowly penetrating the industrial market. The EPA now requires smokestack monitoring of such combustion products as nitrous oxides, sulfur oxides, and carbon monoxide. Reliable chemical sensors that can operate at the high temperatures required in industry do not yet exist for these gases. So, industrial concerns must resort to more exotic means of monitoring—usually infrared (IR) absorption techniques. IR instruments for this purpose are expensive, starting at about \$10,000 each. The competitive advantage of cheaper sensor technologies is obvious.

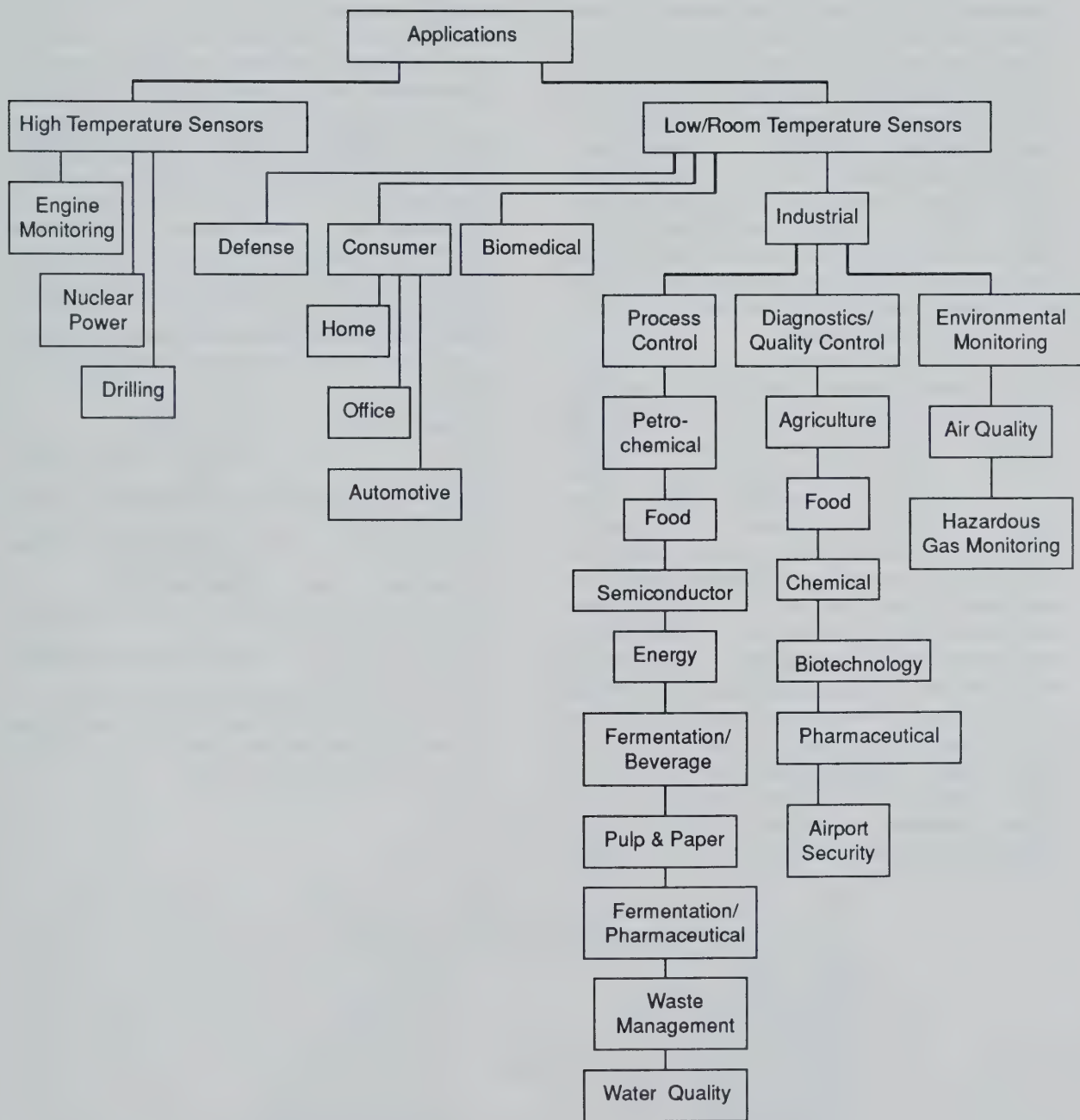


Figure 13. Chemical sensor applications.

4.2.7 Health Care Future biomedical applications of chemical sensors are numerous. For clinical testing purposes, sensors that would replace more laborious immunoassay techniques would be extremely valuable, as would sensors for a variety of proteins, enzymes, and antigens used as diagnostic measures of a patient's condition. Other clinical testing applications that will be very attractive in coming years are sensors to detect low levels of certain drugs in urine. The different sensors for these applications will require a considerable amount of unique engineering, and many of these market niches will be quite small. The development of such sensors will require extensive work, though some of them should start appearing in five years. Sensors that would command a much wider market would include in vivo glucose sensors to continuously control the insulin injections of diabetics and catheter-mounted drug-sensitive devices to help optimize drug-injection rates as well as certain groups of immunosensors such as immunosensors for pregnancy, VD, and theophiline.

4.2.8 Food and Beverage Processing Applications of chemical sensors in the food and beverage industry will be extremely diverse, but certain common ones stand out. For example, continuous pH monitoring during various processing steps would be extremely valuable. Another valuable device would be a sulfur dioxide sensor. Sulfur dioxide is used as a preservative in many foods and in beverages such as wine, where a minimum useful concentration is important to maintaining proper flavor and aroma. Chemical sensors for monitoring the fermentation of wines, beers, and alcohols would improve product uniformity and reduce costs. Devices for this purpose would include sensors for glucose, fructose, ethyl alcohol, pH, dissolved oxygen, dissolved carbon dioxide, malic acid, and tartaric acid. Other general-purpose sensors include potassium-ion sensors, sodium sensors, chloride sensors, and calcium sensors. Although

many of the latter chemical sensors exist in one form or another, they currently are not very useful in the food-processing business because of surface contamination problems and high maintenance requirements.

In table 3, we present a list of some companies involved in chemical sensor research.

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Table 3. Selected Companies Involved in Chemical Sensor Research

Abbott Laboratories	Honeywell Inc.
Allied-Signal Inc.	ITT Corp.
Robert Bosch GmbH (Europe)	Menoto (Japan)
Denki Onkyo (Japan)	Motorola, Inc.
Dow Chemical Co.	New Cosmos Electric Co.
E.I. du Pont de Nemours & Co. Ltd. (Japan)	
Ford Motor Co.	Phillips Petroleum Co.
General Electric Co.	Siemens AG (Europe)
General Motors Corp.	Thomson-CSF (Europe)
Hitachi, Ltd. (Japan)	Toshiba Corp. (Japan)

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The Challenges and Opportunities of Sensor Technology

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Sensors find applications in a wide variety of areas such as medicine, biotechnology, food, automobile industries, and defense. However, their commercialization has been slow. This chapter discusses the challenges which have to be overcome before sensors that work in the laboratory become useful in the real world such as hospitals, plants, or other work places. One main problem in the sensor development has been (and still is) the communication gap between different branches of science. The difficulty

has been the communication of chemical, electrochemical, and analytical spectroscopic ideas to engineers and physicists. The breakdown of barriers between different disciplines will greatly facilitate the development of better quality sensors and open up new opportunities in several areas.

Key words: biosensors; challenges; chemical; communication; electrochemical; opportunities; optical; selectivity; sensors; stability.

1. Introduction

Challenges of sensor technology occur in three broad areas:

- Improving communications among basic scientists (concepts), materials scientists-engineers (development and technology), and users-applied sciences, medical sciences, biological sciences and regular consumers (applications)
- Developing new technologies for macro- and microchemical and physical sensors using classical principles and effects from optics (geometric and physical), electrochemistry (mainly electrostatics and transport), biological sciences (chemistry of biological reactions including transport), and mechanics often with electro- or magnetomechanical and thermal effects (physical measurements)
- Solving user-related application problems of general and specific natures—a membrane cell or a battery either chemical or physical vibration, force,

displacement, velocity, acceleration, flow rate, volume, time, 3-D structure that is, an optical absorption change requires a probing light beam. A resistance change requires an applied AC voltage, etc. Temperature changes from background; measurement of position, angle, optical transmission, absorption, refraction, and emission; and scattering changes, magnetic permeability changes, and voltage, current, resistance, and capacitance changes are most often the transduction part of chemical and physical sensor devices.

2. Proposed Definitions: Chemical Sensors and Biosensors

Chemical sensors are sensors that use chemical processes in the recognition and transduction steps. Biosensors are a subsection of chemical sensors that use biological recognition processes. Both are intrinsically chemical.

3. Why Sensors Now? Challenging the Communications Barriers

Analytical chemists have done research on and made and used sensors for years. Ion-sensitive and redox-sensitive electrodes have been known and used for 100 years often involving sensors reviews so numerous that one out of every three or four sensor papers is a review! Janata has also written a comprehensive, readable, introductory book [34]. The volume edited by Edmonds [35] is very good but requires more familiarity with the field. Comprehensive treatment and many useful examples of modern sensors are in Dessy's work and in his reviews [36].

3.1 A "Chemical" Challenge for Solid-State Technology

By the mid-1960s, commercial bipolar and field effect transistor production using planar, photolithographic, batch processing dominated the electronic device market. Acid-resisting coatings developed by chemists pushed this technology, and microelectronics became the basis for integrated-circuit developments [37–39]. Piezoelectric pressure sensors appeared in about 1962 [40], and microelectronic versions soon followed [41].

Given the unbridled enthusiasm for chemical sensors using solid-state technology, two fundamental problems appeared that were not immediately appreciated:

- Ease of fabrication and large markets for electronic data processing chips do not translate into easily made, inexpensive, large-volume chemical sensors,
- Difficulties exist in making stable microfabricated sensors for real, hostile chemical environments.

After 1970 and the discovery of the prototype chemically sensitive semiconductor device [42], electrical engineers and solid-state physicists took up the chemical sensor field while continuing the physical sensor developments (see the Appendix). Their contributions have been decisive in micro-miniaturization—especially in the fabrication of small versions of already practical devices [43].

Microfabrication is not a panacea in the sensor field. Applications of the field effect-based sensors per se in microfabricated devices seem very much less important than researchers thought at the time of the first enthusiastic conference on chemically

sensitive field effect semiconductor devices [44]. For example, excellent potentiometric and amperometric microsensors can be made on SiO_2 - or on Si_3N_4 -passivated silicon chips—using photolithography and microfabrication, but without use of the field effect [45]. On the other hand, Faradaic processes are susceptible to microminiaturization as well as the nonfaradaic, blocked electrode, field-effect processes. These issues were a theme at the 1980 NATO ASI on Chemically Sensitive Electronic Devices [46]. The challenge to solid-state technology is the development of proper chemical recognition steps and processes in the overall microsensor design. Here is a clear example of the opportunities from matching chemistry with physics and physicists with chemists to make a superior product.

3.2 Opportunities for Physicists and Chemists Working Together

The contributions of chemists and physicists in designing quartz-crystal piezoelectric devices have expanded the chemical-sensor field greatly, although the concept of mass sensors for ambient adsorb- and absorbable gases using the change in resonant frequency of a bulk quartz crystal was exploited some 20 years ago [47,48]. The forms of devices—thick crystal bulk wave (conventional gas analyzer or film thickness monitor) [47], thick crystal shear wave (quartz microbalance) [49,50], SAW delay time (Rayleigh wave along surface of a relatively thick crystal exposed to gases or liquids) [51], SAW resonant frequency excited from the under side of the crystal [52,53], and very thin quartz films oscillating in complex ways (Lamb waves) [54]—are parts of the emerging technology for applications to gas and liquid samples (see review issue [55]).

Here is a challenge, an opportunity for physicists and chemists working together, to combine the recognition chemistry of surface films with the underlying sensitivity of piezoelectric materials. Materials with crystals in 20 of the 21 space groups without a center of symmetry show changes of dimension with application of an electric field. Conversely, application of pressure to effect a dimension change generates an electric field. These piezoelectric materials include the relatively rare group of materials, ferroelectrics, whose crystals show oriented dipoles (or equivalent charge separations) that are not internally compensated. Above a critical temperature, the Curie Point, the effects go away with anomalies in dielectric con-

stant and heat capacity. A curious piece of nomenclature has arisen in this field. In principle, the many devices with field-generated oscillations should be called "electrostriction" devices. Application of pressure to generate electric fields are properly piezoelectric.

Another challenging area of joint research is the development of chemical sensors using thermal measurement, made possible by pyroelectric materials. On the same side of the irreversible thermodynamic equation as the piezoelectric effect is the pyroelectric effect in which a thermal gradient induces a field and a measurable voltage drop [56,57]. This latter effect is presently used in the DC mode, in contrast with the piezoelectric devices that use the AC mode. Many of the same materials show both pyro- and piezoelectric effects. In fact, all pyroelectric materials are piezoelectric, but the converse is not true. However the rules for selecting materials require knowledge of crystal symmetry. Unfortunately, silica and GaAs, while piezoelectric, are not pyroelectric. LiTaO₃, BaTiO₃, and lead zirconium titanate (PZT) are interesting examples of pyroelectrics because they have nearly constant pyroelectric coefficients over a wide absolute temperature range. The latter is crucial for relating heat flux to voltage or current in a simple way. An exciting, relatively nepyroelectric material is polyvinylidene fluoride (PVF).

Microfabricated devices, including pyroelectrics and stacked thermocouples using Cr/Au or Si p-n junctions, are aimed at temperature sensing (to identify phase transitions, as in DTA, although originally they were used for radiation detection), or precision adiabatic temperature changes to determine thermal energies (calorimetry or enthalpimetry). These new devices are exceedingly sensitive to thermal gradients (heat flux or temperature differences of 0.1 mC degree or 0.176 mW/cm²) and seem, at this early date, to be a most profitable direction for chemical sensors using thermal effects: adsorption-desorption, solution-dissolution, simple bond formation (of all types) dissociation enthalpies [58,59]. This statement does criticize temperature sensors such as thermistors, pellistors, and thin metal films, whose physics and chemistry remain an interesting field for research on mechanisms of charge conduction and material properties.

A new and curious sensor type may be related to the field emission or to other surface phenomena leading to currents across space between relatively low-voltage electrodes. Already in the fields of spark/arc spectroscopy and spark source mass spec-

troscopy, certain low-voltage currents, after an initial high-voltage induction process, were found. Reactions of generated charged and neutral species with ambient, usually residual gases were expected and found [60]. Micro spaced electrodes, probably with rough high-field-generating protrusions have been found to make gas sensors [61] and have been used as gas-chromatography detectors [62]. These presumed field-emission-effect electrodes have been waiting for applications and explorations by chemists for many years.

In the past several years, microredox electrodes have been found to produce steady currents under mass-transfer-controlled conditions. There is intense effort to make and understand transport phenomena at single-fiber electrodes of sub-10 micron diameter [63]. However these microelectrodes are inert—for example, carbon, gold, or platinum—and may be small disks, hemispheres, or lines. Although eminently important for sensing currents that are proportional to redox species, notably neurotransmitters, these microelectrodes are not the topics for this set of sensor examples. They are closely related to the whole subject of redox electrochemistry, and the devices may, or may not, be used as sensors.

The use of electron work-function measurements as a sensor opened another research area of common interest to chemists and physicists [34]. The vibrating capacitor (kelvin probe) has been known for 100 years [64], but the significance of the apparent work-function values of phases has been a controversial topic in electrochemistry and electrochemical physics. Ideally, clean, flat materials (usually clean metals, but the category can include liquids), separated by an ideal insulator (usually a vacuum), can be electrically characterized by vibrating them with respect to each other, while nulling the AC voltage. Ignoring fringing fields, the null condition is assumed to be the zero charge, flat-band condition, and the DC voltage required for null is the difference between the electron work functions of the two phases. Real systems, even single crystals, are more complicated because the dipole pd is different at each face; irregular geometries make the null condition uncertain; zero net charge may not mean zero charge at each point of the surface. Residual dipoles (at zero net charge) have residual fields that cannot be completely nulled. The size and shape of the counter electrode are equally important. Only apparent work functions can be evaluated for vibrating samples and reference electrodes of irregular shapes and complicated relative orientations.

However, empirical use of work-function changes of a test metal such as Pd in contact with varying ambient partial pressures of hydrogen has been investigated [65]. As long as the system contains one net capacitor the cells can be electrically rather than mechanically modulated and the work function differences (Pd less metal) can be measured. This value depends on the gas composition, because the work function of Pd is altered by dissolved hydrogen. The suspended gate field effect transistor is a more general version that allows gas adsorption selectivities to be used: metal/selective absorbent/gas/SiO₂/metal [66].

The role of optical physics cannot be underestimated for the development of optical absorbance change or luminescence sensors interrogated by probe beams and/or read by emitted light through "light pipes" addressable potentiometry [79,80]. Whatever the interfacial pd is, it can be perturbed and in some sense measured by generation of a charge that partially cancels the local field. Transient capacitive currents are followed that relate directly to the local electrostatic field determined, in part, by the local chemistry.

3.3 Challenges to Electroanalytical Chemistry

For some 20 years scientists have known and exploited many of the current ideas of sensor chemistry. These ideas include the use of chemically modified redox electrode surfaces (the Michael Sharp radical salt electrodes) [81–83]; the use of enzymes in reactive layers to generate, from neutral charge substrate, species that can be sensed (potentiometric enzyme electrodes) [84]; the use of enzymes and inorganic mediators, such as iodide, in potentiometric glucose sensors [85]; the use of enzymes in amperometric sensors (modifications of Clark oxygen electrodes) [86]; and the use of mediators in redox reactions, formerly called "secondary intermediates" in the early 1950s (Br₂-Br⁻ for oxidation of electrochemically inert species [87; see also 10], or Mn^{+2/3} for NO₂ or nitrite [88]). Further examples include the use of selective reagents to generate response specificity—for example, crown ethers and natural and synthetic ionophores in monovalent and divalent ion sensors dating back to 1965/1966 [89]; and, the use of selective layers to extract or partition species into surface layers on sensors has a long history connected with gas, liquid, and affinity chromatographies.

The new technology, contributed by chemists in recent years, has frequently been a perturbation on existing classical ideas, formats, or systems. Elec-

tronic-conducting electrodes with surfaces modified with pure ion conductors (especially natural and synthetic ion exchangers) cannot serve as sensors for nonredox ions because the membrane/electrode interface is blocked (capacitively coupled) [for an earlier analysis of this effect, see 90]. However, there is one new application of this "mismatched" interface system. In the same way that mercury films can accumulate trace metals prior to anodic stripping, ion-exchange films can accumulate trace ionic redox species that could be subsequently determined by either anionic or cathodic stripping [91]. Electronic-conducting electrodes, modified with mixed conductors, can be sensors of organic gases that dissolve and increase ion or electron mobilities in a reproducible way [92].

More important, catalytic layers on electronic conductors provide many new embodiments for sensors, especially amperometric sensors, using enzymes and coenzymes that are not rapid and reversibly electroactive on bare conducting electrodes. Catalysis of electron and proton transfers was already known to Heyrovsky more than 50 years ago. Mairanovskii contributed and summarized that field [93]. Redox enzymes alone often need catalysis by "promoter" films [94]. Adsorbed amine surface films (for catalysis of cytochrome-c) are attributed to Eddowes and Hill [95,96] while organic metal 'promoters' (in this context) are attributed to Kulys [97]. Promotion by compounds such as N-methylphenazinium methosulfate have a long history in biochemistry [98]. Mixed conductor films, such as fixed metal-site redox ion exchangers, may selectively catalyze the surface redox reactions of biologically important species that are not themselves electroactive in rapid, reversible ways [99]. Alternatively, enzyme/coenzymes can be incorporated in mixed conductor films, such as polypyrrole, with exciting applications to amperometry of electrochemically inert substrates such as glucose and ethanol. At this time, there are hundreds of papers on these topics.

The question of just how effective the enzyme system can be made remains unanswered. How many turnovers can the enzyme system, mediator/promoter/enzyme combination sustain? Layered systems of electrode, electron transfer promoter layer (NMP TCNQ-type, ferrocene and ferrocene derivatives, as covalently attached layers or simply suspended or dissolved with different degrees of flexibility in penetrating to the enzyme-active site), enzyme/coenzyme reaction layer, dialysis stabilizing layer, may be less effective than mixed or wired systems. However there are very good measure-

ments of catalytic currents for some of these layered systems [100]. Building an electronic conductor into enzymes to facilitate electron transfer to electrodes seemed an unlikely approach, but proved to be successful [101]. In further work, the role of the covalently attached electron transfer reagents in the modified enzyme was improved using so-called three-dimensional wiring [102] by which the modified enzyme is embedded in a mixed-conductor polymer.

There are many opportunities for small companies in the amperometric sensor field. The ExacTech enzyme electrode for glucose and a series of other commercialized glucose sensors (Yellow Springs Instruments, Yellow Springs, Ohio, marketed by Eppendorf in Europe, Ampliphon in Italy) seems to be very successful. Closed-loop sensing followed by application of insulin, still remains a challenging problem, even with improved glucose sensors. Academic groups such as that of N. Van Rouy at Neuchatel and M. Mascini in Florence have close ties with marketing companies. Likewise there is keen competition on design of immunosensors using enzymes, redox groups, or fluorescing groups as labels. Some major contributors include the Sensor Group at Twente University (Enschede, Netherlands) and ASI, Ltd. [103].

Design of open chain and cyclic ionophores for selective extraction of particular ions was achieved by 1974 and has remained a major accomplishment in the synthetic part of chemical-sensor research [104]. Even the synthesis of crown ethers, that occurred earlier than 1974, has had less impact on the sensor field. Not only have new ion-dipole compounds been made (with controlled locations of ether oxygens, carbonyl oxygens, and nitrogen centers), but new carriers with reversible covalent-bond formation have been discovered [105]. The well-known carbonate electrode proves to be an early example [106]. The major thrust has been to remove ordinary electrostatic (simple ion-exchange) selectivity as the main operating principle that leads to the usual Hofmeister selectivity series. Instead, selective interactions are emphasized to produce sensors that deviate from Hofmeister sequence to permit analysis of phosphate and bicarbonate—including environmental, biochemical, biotechnological, clinical, and medical applications but may not prove to be any stabler or more selective, or sensitive than more usual sensors.

Appendix I

Sensors Versus Systems: Selectivity, Sensitivity, Amplification

Selectivity is a measure of a sensor's ability to discriminate among species of similar chemical character. It is connected with the sensor's primary recognition function. On the other hand, the inherent transduction possesses some amplification, which is further enhanced by the measuring circuit. Thus, sensitivity, which is the measure of low-level detectability is connected with amplification. Some sensors have the amplification on-board. But as soon as one attempts to improve selectivity or sensitivity by additional separation steps or additional hardware, the sensor becomes a system. Once again there can be gray areas when distinguishing sensors from sensor systems. Often it is perfectly clear—for example, when a whole chromatographic system contains a sensor at a distinguishable point.

Sensors, Detectors, Actuators, and Dosimeters

Sensors are chemically reversible devices. When an ambient is presented to the sensor, the interfacial processes are rapid and reversible. Ideally, a response to a step up in measured quantity reaches a steady-state value as soon as the external surface concentration reaches the bulk value by usual transport processes. A step down returns the response to a reproducible lesser value or baseline. Continuous cycling of steps up and steps down with different magnitudes should develop a steady-state calibration response. Some level of hysteresis must be expected and a slow move of base-line response may be tolerated and corrected by sequential standard samples. Some sensors are not reversible and, in the worst case, are one-time detectors that self-destruct or become insensitive after a single measurement. Actuators are sensors or detectors combined with an active output. Dosimeters are virtually unsaturable, irreversible detectors that continue to accumulate a one-directional input signal.

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Metal Oxide Semiconductor Sensors

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Metal-oxide semiconductors, particularly SnO_2 can be used to construct a low cost sensor for combustible gases. Commercial sensors of this type are available. This chapter describes their construction, with the reasons for the form of the sensor and for the use of the various components. The sensor is adequate for many uses, but improvements would be desirable in the areas of

production techniques, sensitivity, reproducibility, stability, and selectivity. This chapter describes some of the current exploratory studies in these areas and suggests possible new approaches.

Key words: catalysts for sensors; ceramic sensors; gas sensors; low-cost sensors; semiconductor sensors; Taguchi; thin-film sensors; tin-oxide sensors.

1. Introduction

Metal-oxide semiconductor sensors are commercially available for detection of combustible gases. They have the advantages that they are low cost, have low power consumption, and provide a signal in an easy form to use (a simple resistance change). They have the disadvantages that they are nonreproducible, nonspecific and unstable, each to a certain degree. Despite the problems, they have a secure niche in the market, and as the problems are overcome by research and ingenious ideas, the market will broaden enormously.

Right now the market seems to be mostly household, trailer, and boat use for the detection of propane and CO. Many industrial applications are being explored, for some of which the present devices are doubtlessly adequate, but the author of this chapter mostly gets calls complaining about their inadequacy and inquiries about what can be done to make them adequate.

2. Tin-Oxide Sensor

2.1 The Theory of Operation of the Tin-Oxide Sensor

The most popular commercial form for the sensors is the Taguchi sensor marketed by Figaro in Japan. We will examine the Taguchi tin-oxide sensor and with this example discuss both the mode of operation and some of the blems to be expected in this and all semiconducting oxide sensors. In the Taguchi type of sensor, the sensor is coated on a ceramic tube, inside of which is a heater (the devices operate at about 350°C). Outside the ceramic tube is a coating of SnO_2 powder, prepared by coating the tube with a paste including the SnO_2 , a binder, perhaps other materials for filtering, and Pd as a catalyst. Gold electrodes are deposited on the tube for electrical contacts for measuring the resistance of the SnO_2 . The device is calcined to a high temperature to set the paste and provide some sintering for the tin oxide. Figure 14 illustrates the form of the device. Figure 15 provides an example of the type of preparation processes in the manufacture.

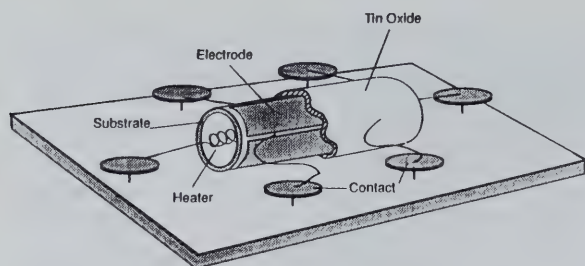
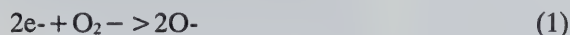


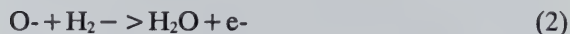
Figure 14. Form of the tin-oxide sensor.

In operation, the heated SnO_2 adsorbs oxygen, presumably in the form of O^- as illustrated in figure 16. The tin oxide is an n-type semiconductor, which means it conducts electricity by means of a relatively low density (compared to metals) of electrons. These electrons are lost to adsorbing oxygen to form the O^- , an insulating region (technically termed a depletion region—depleted of electrons) develops near the surface of each grain of powder. A reaction essentially



occurs, where e^- represents an electron from the SnO_2 . Electrons are left, as sketched in figure 16, in the center of each powder grain, but the conductance from grain to grain is severely hampered by the “intergranular contact resistance” associated with the depletion region near the surface. Thus, when measuring the resistance across a layer of tin-oxide particles, the resistance is very high because the atmospheric oxygen and its effect on the intergranular contact resistance.

If there are combustible gases in the atmosphere, the adsorbed O^- can react with these gases according to a reaction such as



where the e^- is sent back to the tin oxide. With the surface electrons restored, the intergranular contact resistance decreases substantially, and the overall conductance of the pellet goes up. It is this increase in the conductance that signals the presence of the combustible gas. Overall, chemically, a steady state is reached between reactions (1) and (2), and the density of electrons in the tin oxide varies, depending on the concentration of the combustible gas such as H_2 .

2.2 The Reasons for the Choice of Materials

Before we can rationalize changes in construction, we first should examine why the present design and the present material were settled on.

For this type of behavior the solid must be a semiconductor, at least near the intergranular contacts. A metal has too many electrons, and too small a fraction will be lost to adsorbing gases to have much effect on the conductance of the metal.

The semiconductor does not necessarily have to be an oxide, but for operation in air at an elevated temperature, other semiconductors will change their characteristics with time because of the formation of an oxide film. For example, in the author's laboratory we tried to use MoS_2 as the semiconductor. This is a relatively stable sulfide. The material seemed to work well until we deposited Pd as an oxidation catalyst to accelerate the resistance changes. Unfortunately the catalyst also accelerated the oxidation of the MoS_2 and the material had to be abandoned because the properties all changed with time. Thus, we believe that unless other considerations are dominating, an oxide semiconductor is needed.

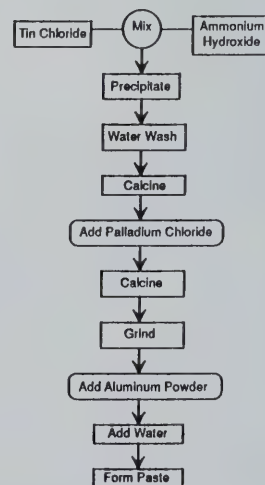


Figure 15. A possible scheme for preparation of the tin-oxide sensor.

Not only does the semiconductor have to be an oxide, it has to be a very stable (or unstable) oxide. (The unstable case is discussed in sec. 4 below). The oxide cannot slowly change its bulk stoichiometry (metal/oxygen ratio) either by reduction, dissociation, or reoxidation. In principle there is a thermodynamic equilibrium in the stoichiometry for each oxide at each temperature and under each

gaseous ambient. For oxides of interest here the approach to equilibrium should be very, very slow at all temperatures and gaseous ambients the oxide may encounter. If the oxide slowly changes its stoichiometry, then it changes its bulk electron density (every oxygen vacancy produced donates a conduction electron), and the coating's resistance varies with time under constant gaseous ambient conditions. The only changes in resistance that should be allowed, for these sensors, are changes due to adsorption/desorption (as discussed above), not changes due to reaction of lattice oxygen.

The oxide semiconductor should be n-type, to measure the partial pressure of combustible gases. If the sample is n-type (conducts by excess electrons) then the resistance will be high in air, due to electron capture by oxygen. The resistance becomes low when the combustible gas is present [eqs (1) and (2)], and this is the direction of resistance change most conveniently monitored. For reasons beyond the scope of this article, the sensitivity is very low if we have a system wherein the resistance increases with the adsorption of the gas of interest. We will assume in this article that the semiconductor is n-type except when specifically stated otherwise.

The use of intergranular contact potential as the resistance-determining parameter is almost required by sensitivity considerations. In the thin-film sensors (see below), workers have tried to make films thin enough so that the extraction of electrons by the oxygen would have a significant effect on the overall film resistance as indicated in figure 17. In figure 17 the depletion region from which electrons are removed is shown, and the conductance is proportional to the thickness of the remaining conductive oxide. This approach is discussed in section 3.2.

In the ceramic case, the sensitivity comes from the depletion region at the intergranular contact. Although it may cause a minor lowering of the sensitivity, a slight sintering of the tin oxide is apparently desirable. Sintering is the fusion of the grains at high temperature, where the area of the fused contact depends primarily on the temperature reached and secondarily on the time it is held at that temperature. The sintering presumably improves the stability over what would otherwise be a variable intergranular contact sensitive to vibrations, temperature, etc. The sintering must be slight however, to allow the oxygen access to the region of the contact as in figure 16. If the sintering were enough to fuse the particles together to

provide a broad contact area, then again the extraction of electrons by the oxygen would not have the desired effect.

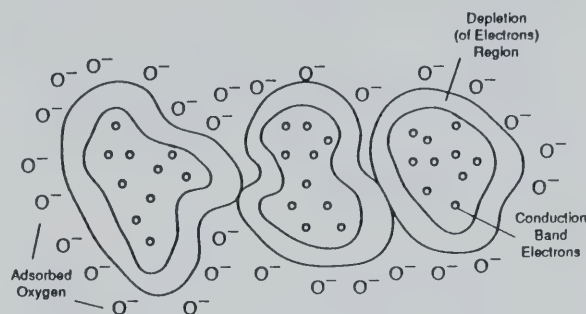


Figure 16. Three grains of SnO₂ are shown, with the conducting electrons indicated and the high resistance depletion region from which the electrons have been captured by the oxygen to form the indicated O⁻.

The catalyst Pd is added to speed up the chemical reaction. Without the catalyst to dissociate the gas molecules (presumably oxygen in particular) the rate of reactions such as reactions (1) and (2) would be much too slow. It would require hours to reach a new steady state as the gas partial pressure is changed. Palladium is presumably preferred over platinum for the Taguchi sensor because of the slight volatility of platinum oxides in this temperature range. Other catalysts such as silver have been tested, as discussed in section 3.1.

Binders are included of course to provide a solid ceramic after calcining (fig. 15), to stabilize the device against changes in contact resistance due to vibrations, etc. Other materials such as SiO₂ have been found to act as a filter or sorbent for incoming gases to help selectivity.

The use of the ceramic tube maximizes the utilization of the heater power, so that all the power is used to heat the tin oxide (as opposed to a flat configuration with the tin oxide on one surface, where some of the power is wasted). It also simplified the form of the heater wire, which can be a coil inserted into the tube.

Given these factors, it is clear to some extent why the sensor is designed as it is. It is also clear, noting the messy procedures, how the problems with the Taguchi sensor arise. In subsections 2.3 to 2.8 we discuss the problems in more detail. I suspect that readers will tend to throw up their hands in despair by the time they have finished section 2, but they must keep in mind that there already is a significant market for these devices and any improvement will broaden the market.

2.3 Production Problems

First, noting figures 14 and 15, it is clear that the design is not suited to mass production. The design of figure 14 leads to excessive hand labor. Many studies of the so-called thick-film sensor discussed in section 3.2 are in part intended to offer more automated production such as screen printing for production of the tin-oxide films. But even then the whole process must involve hand labor and its resulting irreproducibility.

2.4 Reproducibility of Characteristics

The design is not conducive to reproducibility in any way. Compressing and sintering a ceramic coating while expecting reproducible electrical properties is asking too much. Undoubtedly there will be favored paths for the current through the powder mass, and each sensor will be significantly different. The initial resistance value under a calibration atmosphere (e.g., 100 ppm propane in air) will differ from sample to sample because of random differences in intergranular contact resistance. Part of this problem may be variations in defects and impurities (doping) from grain to grain. The concentration of sorbed oxygen (with no combustible gas present) will vary with the concentration of defects and dopants. This concentration will in turn affect the rate of reaction (2) and will therefore have some influence on the signal. The grains are not (to this author's knowledge) intentionally doped to control the conductivity as silicon is. The deposition of the catalyst (e.g., the size of Pd crystallites and how close they are to intergranular contacts) will vary from one time to another. The disposition of binders and other ceramics in the coating may vary. Differences by a factor of 5 in the resistance under identical external measuring conditions and 100 ppm combustible gas are not unreasonable. The problem can be alleviated by designing external circuitry that can be adjusted for each sensor to give a constant sensor output in the 100-ppm atmosphere.

Other sources of random characteristics are possible. The pores through which the gas must penetrate to reach the surface of each grain will be different from sample to sample and lead to irreproducibility. Large molecules may not reach some of the intergranular contacts, while small molecules do. Such differences may account for the unfortunate feature encountered by the author in tests where by external circuitry a few SnO₂ sensors were adjusted to give the same voltage output un-

der 50-ppm propane, but the sensor so adjusted gave wildly variant (factors of 3) differences when the voltage was measured under 50 ppm CO.

A problem in catalysis that may provide a reproducibility problem here is the movement of catalyst atoms at high temperature. If the calcining temperature is high, the effectiveness of the catalyst may decrease as small particles of the catalyst join to make less effective big particles. In the catalytic literature this is termed sintering (not to be confused with sintering the ceramic to fuse contact areas), and it occurs rapidly for noble metal catalysts at temperatures upward of 600 °C.

2.5 The Sensitivity of the Tin-Oxide Sensor

As discussed above, the sensitivity of the sensor arises because the electrical current must pass through the insulating region at the surface of the grains. Thus any small changes in oxygen adsorbed lead to relatively large changes in resistance. Typically, the resistance changes by about a factor of 10 upon adding 100 ppm of combustible gas to dry pure air. Normally, however, one calibrates the sensor with a gas mixture, not with pure air, as the resistance in pure air is relatively poorly defined. Therefore a better way to state the sensitivity is to say that typically it is sensitive to about 10 ppm of combustible gas, and the resistance change varies approximately as the square root of the partial pressure of the combustible gas. This is an adequate sensitivity for many purposes. However, higher sensitivity devices are always desirable.

2.6 Stability of the Tin-Oxide Sensors

The design is not conducive to stability of signal. One stability "problem" arises if the device is allowed to cool to room temperature (to save power when it is not being used). Apparently, organic vapors adsorb on Pd catalyst and possibly on the tin-oxide. When the device is turned back on, immediately upon reaching temperature this material is combusted, leading to a huge false signal. Part of the problem with a "huge" signal is that parts of the device that are normally inactive because they have a long time constant for one reason or another are affected. Then the return to steady state for these portions can be slow. The main false signal lasts a few minutes, but it can take days to return to a baseline where the limit of sensitivity and accuracy is reached, so if this limit is needed, the device must be kept hot always. On the other hand, the false signal (after the sensor has

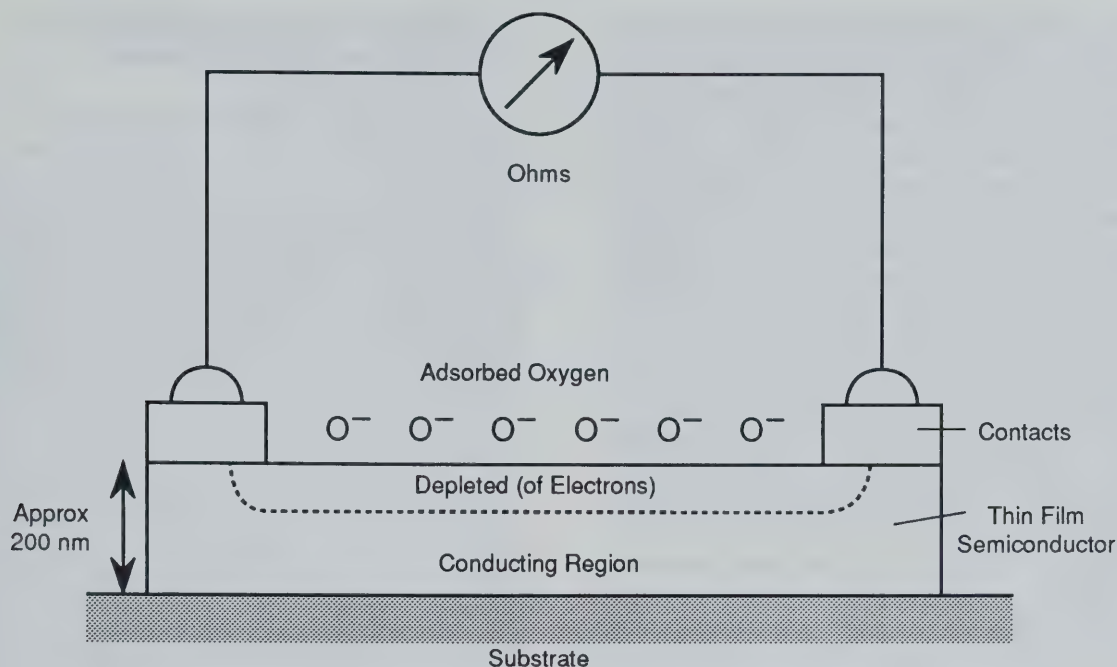


Figure 17. A thin-film n-type semiconductor where the depletion layer is a large fraction of the sample thickness.

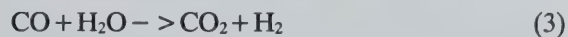
been cool) may not be a problem in certain applications, such as testing for spilled gasoline or propane in the bilge of a boat. Here the false signal when turning the sensor on shows that the gas-monitoring system is working, a good feature. And because the highest accuracy is not required the circuit can be adjusted so that after a minute or so of waiting the signal disappears (if the bilge is safe).

The author has seen no test results for the response after an overloading pulse of combustible gases, but suspects that the characteristics will be much like those in the false signal case—namely, requiring time to recover.

Other stability problems involve a slow variation after the device is manufactured. System manufacturers who want good accuracy run the device under normal operating conditions for the order of four days or more before calibrating the device, and then urge those users who demand high accuracy also to run the device 4 days before they consider the device “stabilized.”

A surprising degree of instability (a factor of 2 in resistance) has been reported resulting from room-temperature changes. As the heater current is kept constant, some variation in the temperature of the sensor is expected as the ambient temperature varies, but that seems high. No other reports have been found to compare.

Stability problems because of fluctuations in relative humidity (RH) are present. In the case of measurement of CO, the problem is particularly acute, because a water-gas shift reaction



can lead to the sensor's combusting hydrogen instead of CO, and the sensors have quite a different sensitivity to the two gases. Thus the signal depends on the H₂O concentration in the air. The same problem may appear in the detection of organic compounds if CO is an intermediate in their oxidation steps. Certainly it has been shown that water vapor suppresses the reactivity of hydrocarbons on noble metals. Other influences of water vapor are not so clear. It has been known for many years that water acts as a donor, injecting electrons into the conduction band of semiconductors. This may be one of the RH problems.

There are other possibilities for stability problems. One is the stoichiometry problem discussed above, where it was pointed out that if the semiconducting oxide changed its stoichiometry near the surface problems instability would arise. The ceramic is calcined at high temperature during the device preparation, and it may be that conditions are favorable for dissociation of the oxide near the surface—the tin/oxygen stoichiometry changes to

SnO_{2-x} (of course the tin-oxide can be reoxidized by heating in oxygen at an intermediate temperature, but this adds a complex production step). Then the material is stored at room temperature for a long period of time. At room temperature, the stoichiometry should be close to SnO_2 at equilibrium, but the rates are very very slow. But some reoxidation near the surface could occur. Heating again to 300–350 °C for operation, the equilibrium stoichiometry is again different, and the stoichiometry will very slowly change. The point is that these slow changes in stoichiometry are followed with changes in bulk resistivity that show up as a drift in the overall resistivity and sensitivity of the sample. Other models for long-term drifts are the slow evolution of adsorbed water vapor or grain growth of SnO_2 .

Poisoning of the surface, via a strong reaction at the surface by some active gas from the atmosphere, can give rise to stability problems, in the limit to where the sensor becomes dead. Silicone oils, lead, freons, and sulfides are examples of poisons that will deactivate the catalyst.

2.7 Selectivity, the Discrimination Between Various Combustible Gases

The greatest problem for many applications of the semiconductor sensor for combustible gases is that it responds to all combustible gases. Any gas that will react exothermally with oxygen to produce CO_2 or H_2O will burn on the Pd catalyst and produce a signal. For general gas detection, we would prefer to have available a choice of selective sensors: one sensitive to CO and nothing else, one sensitive to H_2 and nothing else, one sensitive to CH_4 and nothing else, and so on through the list of possibilities. But that is not available in the semiconductor-oxide sensors. In section 3.1 we discuss some of the techniques used for the Taguchi-type and other oxide sensors to introduce some selectivity.

2.8 Power Dissipation

The unit as constructed in the figure 14 example requires a power of about 1 W, primarily to maintain the 300–350 °C temperature needed for the catalytic reaction. When batteries are used to provide the power, which is common for many applications, this becomes a problem.

3. Example Techniques Studied for Improvement of the Sensor Characteristics

3.1 Catalysis and Other Ways to Improve Selectivity

Some selectivity can be induced in the sensors. Unfortunately none produces the ideal selectivity desired. Figaro advertises a series of sensors designed to emphasize various gases. For those interested in improving sensors, especially with respect to selectivity, the Figaro catalogs should be examined and, to the extent possible, the variations in the composition of the SnO_2 -based ceramic examined that lead to the modest selectivity.

The catalyst chosen determines selectivity to some extent, for one species will react faster on a given catalyst than another. Studies of catalysts and promoters for gas sensors are appearing more in the literature. A “promoter” is a species added that is not a catalyst itself but makes the catalyst work better. For example in hydrodesulfurization catalysis, the removal of sulfur from oil, the catalyst is MoS_2 . But one obtains much greater activity with Co added, even though the Co by itself does almost nothing.

In principle the catalytic literature should be perused in great detail to provide ideas about better catalysts and promoters. The trouble to date has been that the catalytic literature has emphasized catalysts where a known gas mixture goes over the catalyst and the selectivity desired is in the product of the catalytic reaction. For gas sensors, one does not care about the product at all. One wants certain gases in a mixture to be burned on the catalyst selectively over others. So the catalytic literature is only of moderate value. This gas-sensor business, however, is certainly an area where someone knowledgeable in catalysis could make a significant leap forward.

Examples of catalyst changes that have been studied are variations from simple approaches such as exposing the sample to SO_2 at 500 °C (which is reported to make a significant change in the sensor selectivity), to changes in the dominating catalyst from Pd to Ni, Ag, Au, Cu, Pt, Nb, V, Mo, Sb, and Th. The authors claim changes in the selectivity for these various modifications, but in many cases the basis for the claim is not explained. For example, an author may say he has developed “a CO sensor using SnO_2 with catalyst X” but only show results for CO, showing that it responds to CO but not that it is selective to CO. In any case, by the nature

of catalysis, one will never attain almost perfect selectivity by modification of the catalyst/promoter system.

Filters or catalysts to remove unwanted gases before they reach the tin-oxide have been studied. The potential disadvantage is the consumption of the gas of interest by the filter or the catalyst. Zeolites, to block large poison molecules, or other microporous materials have been used. Teflon to block water, carbon to block NO_x , and even SnO_2 to block methanol have been reported. The problem, as mentioned before, is to avoid blocking gases of interest. For methane specificity, other gases have been oxidized from the atmosphere by a Pt catalyst before the carrier gas reaches the sensor. Because methane burns at the highest temperature, this can be done.

Temperature cycling of the sensor has been investigated. Some gases are burned at low temperature (e.g., CO), some at high temperature (e.g., CH_4) so in principle one gets a series of peaks as one ramps the temperature, especially in a closed package, where one can first burn the CO, then observe the conductance peak, then at higher temperature burn the methane and observe its conductance peak. Cycling the gas in a closed package becomes a problem, for after the ramp, of course, one must exhaust the consumed gases and introduce a new aliquot. In any case the peaks observed during temperature cycling are quite broad, and one gas may show several peaks.

Another approach in temperature cycling is to alternate the temperature between two values. For CO it has been proposed to alternate between a high temperature (e.g., 350°C to burn excess gas adsorbed), then a lower temperature to measure CO, a material that can be oxidized at, say, 100°C . This is discussed further in section 4.5.

3.2 Thick-Film and Thin-Film Sensors

The term thick-film sensors is applied to layers of sintered powder oxide, similar to the Taguchi sensor except that the deposition is usually planar and the semiconductor oxide need not be SnO_2 . The sensors can be prepared by techniques as simple as drying a drop of slurried oxides (+ catalyst) then calcining. We have found that pyrolysis of a spray of $\text{SnCl}_4 + \text{SnF}_2$ solution, such that the grains are formed before deposition on the hot substrate, is effective in producing reasonably uniform grains.

A popular heater for thick-film sensors, deposited as a layer under the active semiconductor, is RuO_2 .

Spray pyrolysis where the SnO_2 is formed upon contact with the substrate produces a film with characteristics closer to those of the thin-film sensors.

Thin-film sensors are usually produced by vacuum techniques and are an entirely different class of sensors. The main motivation for their development is the ability to produce many identical sensors at once and, when produced, the ability to use the photolithographic techniques developed for integrated circuits, to accurately define the geometry. If the bulk defects are immobile, as in SnO there are two possible modes of operation as a combustible gas sensor. The first is with the sample thin enough so the thickness of the region where electrons are depleted (by adsorbed oxygen) is a reasonable fraction of the thickness of the film. Here the conductance is simply proportional to the thickness of the part that still has its quota of electrons (fig. 17). As more oxygen is adsorbed, the depleted part is thicker, and the conductance is lower. When a combustible gas is present, causing injection of electrons, the depleted part is smaller and the conductance is higher. Because the depleted part is on the order of 1000 to 2000 Å, the sample thickness should be on the order of 5000 Å so that there is a reasonable conducting portion. As the conducting region becomes smaller and smaller, the sensitivity becomes greater and greater.

However, there is a sharp limit. If the depleted (of electrons) part extends through the whole thin film, the conductance/combustible gas pressure relationship is not well defined. Studies of thin SnO_2 whiskers have indicated a lowering of sensitivity with very thin samples.

If the depleted part only extends partway through the film, as desired, the sensitivity depends strongly on the film thickness and the doping or defect concentration. For highest sensitivity, the depletion region should extend almost all the way through the sample. The thickness must be accurate so that it does not extend all the way through. And moreover, because the thickness of the depletion region varies as the square root of the doping or defect density, the densities must be accurately controlled—an even greater problem. The sensitivity is normally much lower than that of sintered powders but is quite acceptable.

A problem with thin-film sensors is that the resistance is often not controlled by the bulk electron density but by grain boundaries in the films. In principle, grain boundaries should be avoided—single-crystal films should be used for easy inter-

pretation. But thin (5000 Å) single crystals are not obtainable in the usual crystal-growing techniques, and evaporated or sputtered films inevitably have grain boundaries. Again, intergranular contact resistance is involved in determining the resistance of the film, and, because oxygen cannot directly reach the intergranular contact (as it does in the powder case), it must diffuse in and out at the grain boundary. This makes for long time constants. Blocking the grain boundaries completely (gold deposition was used in one attempt) is a solution if an effective block to O diffusion can be realized.

Thin-film sensors for H₂S detection seem to operate by a different mechanism—that of ion exchange at the surface. A thin layer at the surface (of SnO₂ or WO₃ films) becomes essentially a sulfide, of much higher conductivity than the oxide. In this case the grain boundaries are less important, except possibly to affect their response to combustible gases as interferents. Perhaps because of this, or perhaps because the ion exchange occurs too deep for easy reversibility, the H₂S sensors seem to have the same problems as combustible gas sensors, but to a lesser degree.

3.3 Other n-Type Semiconductors Tested

For some semiconducting oxides, the probable mechanism of operation is different somewhat from that described in section 2.1 for SnO₂. Specifically, the gas of interest can react with lattice oxygen in the sensitive powder instead of adsorbed oxygen. The interaction can be extraction of the oxygen (reduction of the oxide) or ion exchange. In the case of reduction of the oxide, the oxygen vacancy diffusion rate must be either very low (in which case the sample acts just like SnO₂, with oxide oxygen simply substituting for the adsorbed oxygen) or the oxygen vacancy diffusion must be very high—a case of particular interest.

Titanium dioxide and bismuth molybdate are examples where the defect mobility is high, and the operation is based on that fact. As was pointed out in section 2.2, the defect mobility must be either high or low for a stable sensor—that is, the defects must either remain at the surface or diffuse rapidly and evenly through the sample. If the vacancy mobility is in between, there will be unacceptable time drifts in the conductivity as the bulk slowly tries to reach equilibrium with the surface.

This approach—the use of materials, where the lattice oxygen rather than adsorbed oxygen is the reactant, with the combustible gases—could be

used elsewhere, and will be discussed more generally under section 6.4.

Bismuth molybdate (Bi_{2x}Mo_yO_{3x+3y}) has been under study in our group. It is an important commercial partial-oxidation catalyst. Its chief interest to us has been the possibility of operating the sensor at a reasonable temperature (circa 300–350 °C) under the “high-vacancy mobility” mode of operation. It is known from the catalysis literature that oxygen vacancies have an unusually high mobility in these materials. The oxygen-ion vacancies are donors that provide the conduction electrons, so the reaction of the combustible gas, to increase the density of vacancies, increases the bulk conductivity of the material, not just of the intergranular conductivity. We use films of appreciable thickness (1 μm). However, they are thin enough so they can easily change their stoichiometry throughout the whole film. By avoiding the surface problems, we improve reproducibility and stability manyfold. The selectivity favors certain easily oxidizable gases such as alcohols and ketones. To date we have not added a catalyst, and because the material is very insensitive to CO and H₂, there is negligible effect of the humidity.

TiO₂ is under study as an air/fuel ratio sensor, working at high temperature where the lattice oxygen is part of the reaction rather than adsorbed oxygen. The temperature of operation is very high, >600 °C, so that the bulk vacancies are mobile. The extraction of a lattice oxygen from the TiO₂ gives a donor vacancy as with the bismuth molybdate above, and so again we have a sensor depending on bulk behavior. TiO₂ is a case where this author would not expect a useful sensor for low temperature (and hence a low power requirement), because at temperatures on the order of 300 °C, the oxygen in TiO₂ is of intermediate mobility.

ZnO is another sensor with poor diffusion characteristics. It tends to dissociate easily, giving up its lattice oxygen, but the vacancies are relatively immobile. In addition to oxygen vacancies there can be interstitial zinc produced by the reaction of a combustible gas with ZnO, and the interstitial zinc has a high mobility. Thus the material has complicated bulk properties and intermediate mobility defects.

Fe₂O₃ has been offered as a commercial sensor to compete with the Taguchi sensor. It is found that the phase is much more sensitive than the phase of iron oxide. It has been claimed that a Ti-doped Fe₂O₃ with a gold catalyst is selective toward CO compared to H₂ or ethanol.

A sensor based on $\text{In}_2\text{O}_3/\text{CuO}$ (2–25% In) is reportedly selective to propane. WO_3 thin films (6000 Å), Pt activated, have been studied as sensors for N_2H_4 , NH_3 , H_2S , and H_2 .

3.4 The Use of p-Type Semiconductors for Detection of Oxidants

Whereas n-type semiconductors conduct because they have a few excess electrons over what a perfect crystal would have, p-type semiconductors conduct because they have a few electrons missing. The empty sites for electrons are called “holes.” (The defect-free nonmetallic crystal has all energy levels so perfectly occupied that the electrons cannot move; only excess electrons or holes can move and conduct electricity.) With a material that can be p-type (conducting by holes), adsorbing species that extract electrons from the material increase the hole density, and thus increase the conductance. Thus oxidants—such as oxygen, NO_x and halides—will tend to increase the conductivity.

It is difficult to find a material of the oxide semiconductor type that will give a response in air, because normally the huge excess of oxygen in the air has extracted all the electrons reasonably possible, and the conductivity is already high before the NO_x , say, is added. Certainly for this type of a sensor, one does not want a catalyst that will activate oxygen and assist it in electron extraction.

A p-type semiconductor oxide such as this could be found to use in a reducing gas stream for the purpose of detecting small partial pressures of oxygen. The reducing gas, tending to inject electrons, will lower the hole density and cause a high resistance. The presence of oxygen will extract electrons, and the resistance will decrease (the mirror image of the combustible gas sensor).

We have tested NiO doped with Li, a relatively stable p-type semiconductor, for the detection of NO_2 . In this case a surface additive was deposited to enhance the activity with NO_2 and limit the activity of O_2 . The concept of using such additives (together with this example) is discussed in section 6.5.

Phthalocyanine, particularly copper and lead phthalocyanines, has been under investigation as a detector for halides and NO_2 , thus complementing the metal oxide sensors. A reasonably high temperature (about 170 °C) is necessary for a fast response. It is not clear how stable this organic material will be over long periods of time in air at these temperatures. The sensors do not seem commercially available; the problems are not clear.

4. Possible Directions for Improvement

Above we have discussed some of the apparent problems with Taguchi-type sensors. Unfortunately the problems cannot easily be ordered according to dominance. Thus it is difficult to say with confidence what should be improved for a dramatic improvement and what, if improved, will have little effect.

4.1 Mechanization of Production

The great potential advantage of evaporated films is the improvement in reproducibility in production, including the use of photolithographic techniques for contacts as was discussed in section 3.2. However, as also discussed there, other problems appear, such as the reproducibility of sample thickness, the reproducibility of defect and dopant concentrations, and the reproducibility of grain boundaries.

A possible approach is to grow single-crystal films by laser annealing, as has been done with silicon films on SiO_2 . This, of course, depends on melting the material, and SnO_2 may decompose too much before melting. With techniques of deposition such that reasonably sized grains are produced, microlithographic techniques may make it possible to isolate the resistance measurement onto a single grain. Another approach is to block access of the ambient gas to the grain boundaries, perhaps with gold as discussed in section 3.2.

For defect control, accurate doping of the semiconductor may overcome the problem. The use of thick-film technology (screen printing or pyrolytic spraying of very accurately sized grains of the semiconductor of interest) may make production somewhat more routine.

4.2 Alleviation of Reproducibility Problems

When using a sintered oxide powder as the sensor, it seems clear that uniformity of the particles that may lead to uniformity of the intergranular contacts must be the aim. Spherical powder grains would be ideal, if one had a preparation method. Asymmetric particles, a combination of large and small particles, will lead to preferred electrical paths through the ceramic, and the response of the particles on these preferred paths will dominate the device response. If pressure is applied to firm up intergranular contact, it will appear unevenly, and the sintering during calcining will be uneven.

The catalyst should be dispersed evenly over the surface—an improvement more easily achieved. Techniques of catalyst dispersion have been well developed in the field of catalysis, and their techniques could be adopted with possible improvement.

Doping the powder with a donor atom (such as Sb or F) when using SnO_2 , rather than depending on chance (depending on the density of defects in the original material), may help in reproducible resistances for the sensors.

The pore size between grains should be large so the gas has rapid access to all the grains evenly. Larger grain size will both improve the gas exchange and make uniformity of grains easier to achieve, but it makes intentional doping of the SnO_2 more necessary to ensure that contact resistance rather than grain resistance dominates.

Binders that do not block the pores would seemingly be desirable. There is no strong indication that this is a problem except that in some cases the characteristics suddenly grow poorer as the binder/ SnO_2 ratio increases. Pressing the powder together for good SnO_2 - SnO_2 intergranular contact, may help to avoid particle separation by binders or other additives and thus should help make the current paths more uniform.

A parallel array of thin lines such that the electrical current cannot follow a tortuous nonreproducible low-resistance path through the layer, may avoid the problem of preferred paths for the current. This will also yield an automatic averaging of the variations that will help reproducibility.

4.3 Possible Ways to Improved Sensitivity

High sensitivity, as discussed above, depends upon the carriers passing over a space charge region at an intergranular contact or passing through a very thin (circa 4000Å) film. As discussed in the last subsection, techniques should be employed (a) to make the film a single crystal, and (b) to control the thickness very accurately.

There seems to be no good discussion in the literature of the ultimate sensitivity of these devices, but we will argue that it depends at least in part on the rate of electron injection from adsorbed oxygen. With no combustible gas present, the density of oxygen will depend on an adsorption/desorption steady state. The presence of small partial pressure PR amounts of combustible gas will lead to injection of electrons, at a rate perhaps proportional to PR but if this rate is small compared to the adsorption/desorption rate, then the change in the elec-

tron density will be negligible. From this argument, the minimum pressure of combustible gas observable will be that sufficient to inject electrons at a rate approaching the electron-exchange rate for the equilibrium adsorption/desorption of oxygen. Such a model leads to the suggestion that a catalyst is needed that does not dissociate oxygen (so oxygen adsorption is slow) but dissociates combustible gases (e.g., hydrogen). Then the rate of electron injection from the reaction of a combustible gas with adsorbed oxygen may be high compared to the rate of direct-electron injection from O- and the sensitivity to the combustible gas will be high. However, first a good theoretical analysis should be made to confirm the above oversimplified model. A good theoretical analysis will also indicate whether the SnO_2 doping should be high or low, a factor that must come into the sensitivity problem.

In principle, miniaturization will also help sensitivity. A steady-state reaction, wherein the combustible gas is consumed, occurs over each element of area (for a given fractional change in resistance), so the greater the area (summing the area from each grain), the more gas is consumed. If the gas is consumed very rapidly compared to the rate at which it is provided (by convection) the surface concentration will be low and the signal will be low.

For most gases of interest, the sensor operates above T_{max} (as defined in sec. 4.5 below). Above T_{max} the signal will be nonlinear in partial pressure, giving a lower reading than expected by the usual $\text{PR}^{1/2}$ law, because the gas will be consumed in a thin surface layer. A square wave temperature cycle, with the high $T = 350^\circ\text{C}$, the low $T = T_{\text{max}}$ for the gas of interest, will be helpful.

4.4 Stability Improvement

Stoichiometry problems may be avoided by intentional doping of the semiconductor so that the effect on the donor density from variations in stoichiometry will be small compared to the intentional doping. There seems no way to avoid the "huge signal" that appears after the device is stored at room temperature and picks up organic vapors, except either to keep it at high temperature always (requiring power) or to close the device off from the atmosphere (usually too cumbersome, but for special cases it may be worthwhile).

Poisons are a difficult problem. There may be ways (as in catalysis) to remove poisons and recover the catalyst, but these will be individual for the poison. For example, sulfides are removed by hydrogen treatment at 400°C .

The manufacturer of a device could list such procedures for users to follow if they suspect poisoning. The manufacturer could also suggest that a clean device be kept available to replace any suspicious device, for a quick test by, say, alcohol.

The deleterious effects of the water gas shift reaction, (eq 3), are difficult to avoid. Combining a humidity-measuring device with the metal oxide sensor may be practical for systems where only one gas is expected, such as CO. Then an analyzing computer chip could make the correction. Alternatively, and perhaps more practically, research could be done to develop a catalyst that gives the same response to CO as to H₂.

Finally, although it has been mentioned above, one must be careful to choose a semiconductor oxide such that defects are either very mobile or very immobile in the temperature range of operation. Otherwise great instability will result.

4.5 Attacking the Selectivity Problem

If an oxidation catalyst is required to provide good response time, it seems difficult to provide selectivity, because such catalysts normally will catalyze the oxidation of any combustible gas. If one can produce a filter that only allows one species through, such as Pd for hydrogen or stabilized ZrO₂ for oxygen, with characteristics such that it can reasonably be used with the sensor, then this would provide the desired selectivity. But there seems to be nothing in the immediate future that will do this.

It is possible to modify the pattern of response to the various gases by changing the catalyst and/or the promoter. As discussed in section 3.1, there has been considerable effort in this direction. One can, for example, search the literature for oxidation catalysts and promoters and empirically try each system on SnO₂ to check what it does to the selectivity. Varying the oxide also will vary the selectivity. Even varying the dominant crystal face exposed will vary the selectivity. Single crystal ZnO has been shown to have a different selectivity in CO oxidation, relative to other gases, than powdered ZnO.

A particular temperature $T_{\max}$ of maximum sensitivity is found for each gas. If the sensor is held at too low a temperature (significantly below $T_{\max}$) the gas is not oxidized, so there is no signal from the sensor. The mechanism for poorer sensitivity at temperatures above $T_{\max}$ is not clear, but probably arises because the gas is oxidized before it can penetrate into the tin-oxide layer. Then only a thin sur-

face layer shows a conductance change. With this in mind, a good approach to selectivity is to alternate the temperature between a high temperature to oxidize any gases adsorbed on the surface and a low temperature—specifically $T_{\max}$ of the gas of interest.

A possibility for greater specificity lies in the specific chemical reactions that are currently used in such techniques as colorimetry for gas detection and analysis. If these can be deposited on the surface permanently, and if their presence has an effect on the conductivity of the powdered oxide sensor, then they may provide the specificity desired. An example of this tested by the author is sulfanilic acid as a surface additive to the semiconductor NiO:Li (lithium-doped nickel oxide). Sulfanilic acid has a rather specific reaction to NO₂. It is an electron donor in NiO:Li, where the NiO:Li is a p-type semiconductor. Thus, with no NO₂ in the atmosphere, the sulfanilic acid injects electrons and decreases the hole density in the NiO. The resistance is high. When NO₂ is present, its reaction with the sulfanilic acid leads to the electrons being recaptured, and the increased hole density that results leads to a much lower resistance. This combination is not of particular interest as a sensor, at least under the conditions used, because the sulfanilic acid/NO₂ reaction is irreversible, so the device becomes more of an exposure meter than a sensor. But other such specific reactions between a gas of interest and a stable chemical could suggest surface additives for sensor specificity.

The other techniques described in section 3.1 can also be applied. If a unique approach is known to the reader that would not have come to the attention of others in the field, it could be a winner. But it is difficult to suggest others than the ones discussed in that section.

4.6 Minimizing the Power Requirements

The requirement that the sensor be held at 300–350 °C to catalyze the oxidation of the combustible gases leads to about 1 W power dissipation and is a significant deterrent to their use as battery-operated devices. Heat flow from the sensor must be minimized. Miniaturization of the sensor is one approach.

Miniaturization may also have advantages in sensitivity (see sec. 4.3). Silicon micromachining to provide a very thin support for the sensor is another way to decrease heat loss. The concept in the latter approach is to use a silicon chip where, under the sensor material, the silicon has been etched

down to a micrometer or so thick. The cavity etched out, on the opposite side of the silicon from the sensor material, may contain the heater element and be plugged to minimize convective heat flow. Naturally, convection on the sensor side of the silicon "membrane" cannot be avoided. This technique may be too expensive, however.

The simplest way to avoid heat loss is to combine miniaturization with a planar structure on a thermally insulating material such as silica, where the sensor of course must be electrically insulated from the underlying heater.

5. Arrays of Sensors

It is the dream of the future that enough selectivity and stability be provided for these low-cost combustible gas sensors that an array will provide "perfect" selectivity. To determine the concentration of N gases in atmosphere, one should be able to use N sensors, each with a characteristic (and different) response to the gases. Then a simple chip can be designed to indicate the gases present. For a simple example, with two sensors a and b , and two gases of concentration x_1 and x_2 we could ideally have

$$G_a = a_1x_1 + a_2x_2$$

$$G_b = b_1x_1 + b_2x_2$$

where G_a and G_b are the conductances and where the a 's and b 's are the sensitivities to the gases x_1 and x_2 . Knowing the sensitivities a_1 , a_2 , b_1 , and b_2 , the concentrations of x_1 and x_2 are easily calculated. Such an array will provide perfect selectivity, in principle. At present, sensors with the required differences in selectivity are available, using the techniques of section 3.1. However, more research is needed to provide the necessary stability for this approach.

6. Conclusions

The Figaro sensor, based on SnO_2 , is sold commercially, because of its very low cost and convenience of use. It is used despite its problems in reproducibility, stability, and selectivity. Improvements in these areas will lead to an expanded market. Ideas for significant improvement could result in a dominant position in this emerging technology.

Supplementary Reading:

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Amperometric Gas Sensors

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Amperometry is widely used to determine electro-reactive species in liquid and gas phases. This chapter focuses on amperometric gas and vapor sensors. The common characteristic of all commercially available amperometric gas sensors is that measurements are made by recording the current flow in the electrochemical cell between working (or sensing) and counter (or auxiliary) electrodes. An introductory discussion of the general mechanisms of electrochemical gas reactions, the theory of the limiting current, and an outline of the construction of amperometric gas sensors is presented. The performance of sensors and the relationship of the sensor's performance to the materials of construction and sensor components are described. The analytical characteristics of a sensor such as sensitivity, selectivity, response time, and noise are intimately related to the materials chosen for membranes,

conductive electrolytes, and active electrocatalysts, as well as the choice of effective operating conditions (e.g., electrode potentials and sample flow rates). The applications of amperometric gas sensors to specific analytical problems are discussed in the context of required performance. Finally, this chapter points out technical gaps and the future direction of amperometric gas sensor research including some potential applications. References that are relevant to the field of amperometric gas sensors are summarized in a table which contains the author's name, the electrochemical reaction, and an exemplary application.

Key words: amperometric; detection; diffusion; electrocatalysis; electrolytes; gas sensor; membrane; potentiostat; sensor mechanism; toxic gases.

1. Introduction

Amperometry is widely used to determine electroreactive species in liquid and gas phases. This manuscript focuses on amperometric gas and vapor sensors. The amperometric gas sensor is often described in the literature using such terms as *polarographic*, *electrolytic*, *voltammetric*, *amperometric*, *fuel cell* or *micro-fuel cell*. This diverse and sometimes inappropriate nomenclature confuses the method of sensor operation, the description of the sensor design, and the application. Most amperometric gas sensors are not polarographic because they do not contain a mercury electrode. Many are not electrolytic but rather are galvanic cells, and none generates sufficient power to be called a fuel

cell. The major common characteristic of all commercially available amperometric gas sensors is that measurements are made by recording the current flow in the electrochemical cell between working (or sensing) and counter (or auxiliary) electrodes and the working electrode (WE) is a data controlled potential. An appropriate name for this class of sensors is amperometric to distinguish them from potentiometric sensors (wherein the potential at zero current flow is the measured variable or signal) or conductometric sensors (wherein the impedance is measured between two or more electrodes when existed by an external voltage). Such chemical sensors produce a current when ex-

posed to a gas phase containing the electroactive compound because the analyte reacts either producing or consuming electrons within the cell or electrochemical sensor. By choice of a suitable geometry for gas exposure, and electrolyte confinement and appropriate materials for electrocatalysis, as well as electrochemical methods, the sensor can be made quite sensitive for a particular gas component (analyte) and quite insensitive to others.

The following section introduces the general mechanism of electrochemical gas reactions, the theory of the limiting current, and an outline of the construction of amperometric gas sensors. Section 3 reviews the performance of sensors and the relationship of the sensors' performance to the materials of construction and sensor components are reviewed. The characteristics of a sensor—such as sensitivity, selectivity, response time, and noise—are intimately related to the materials chosen for membranes, conductive electrolytes, and active electrocatalysts, as well as the choice of effective operating conditions (e.g., electrode potentials and sample flow rates). Section 4 discusses the application of amperometric gas sensors to specific analytical problems and compares typical performance. Sections 5 and 6 point out technical gaps and the future direction of amperometric-gas-sensor research including some potential applications.

2. Fundamental Concepts of Amperometric Gas Sensors

This section presents some basic concepts about how an amperometric gas sensor is constructed and how it operates. The Clark electrode for oxygen determination is the earliest successful amperometric gas sensor of modern design [1]. This sensor and its fundamentals of operation have been studied extensively [2–6]. The amperometric gas sensor has evolved over the past 20 or more years such that it now exists in many forms. However, all forms share some common characteristics, such as (a) gas entry chamber or membrane, (b) at least two electrodes, and (c) an ion conducting electrolyte. These parts have a large number of possible arrangements, and many choices exist for their composition and preparative methods. This results in a diverse, varied, and rich technology now available for gas sensing. These sensor designs are discussed in a subsequent section; here we shall focus on some common concepts and ideas that will help

us understand this type of sensor no matter what design is utilized.

2.1 General Mechanism of Electrochemical Gas Reactions

A general schematic diagram of an exemplary electrochemical gas sensor reaction (for carbon monoxide) at a single porous electrode is given in figure 18. The response of an amperometric gas sensor can be described by the following nine steps (see fig. 18):

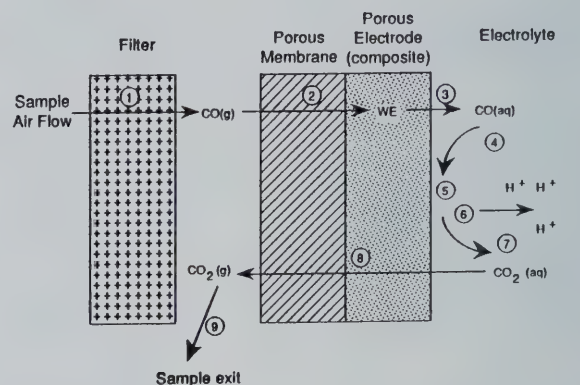


Figure 18. A general schematic diagram of a CO electrochemical gas sensor reaction at a single porous electrode.

(1) *Introduction of the gas or vapor-phase compound to the outer boundary of the sensor.* This can be accomplished either directly or through a filter. The filter can enhance the selectivity of the sensor system by removing unwanted electroactive contaminants and can protect the working electrode area of the sensor from particulate or other contamination. The filter can also be used to react with the analyte and alter its form to a more suitable electroactive species for subsequent detection by the sensor.

(2) *Transfer of the reactant from the gas chamber to the porous membrane in front of the working electrode and diffusion to the electrode/electrolyte interface.* Laminar or diffusion flow characteristics dominate in gas chambers of typical geometries. There may

be a membrane or capillary somewhere between the gas chamber and the electrode. The transfer across the membrane or capillary is usually characterized by a Fickian diffusion expression. The incorporation of any membrane in front of working electrode provides a second opportunity for the sensor engineer to achieve selectivity. A solid membrane in front of the electrode will mean that a permeation process is required for the gas to reach the electrolyte. For example, it is possible to use a thin solid TEFLON membrane to obtain response to H_2 (a gas that rapidly permeates many plastics) and eliminate response to virtually all other analytes.

(3) *Dissolution of the electroactive species in the electrolyte.* The rate of mass transfer across the gas/liquid boundary and the solubility of the gas in the electrolyte dramatically alter the sensor's sensitivity for and response time to a specific analyte. For instance, choice of acidic electrolyte in a hydrazine sensor results in protonation of the hydrazine base and a very different electrochemical reactions. When hydrazine is reacted in a basic electrolyte, the unionized form of hydrazine dominates.

(4) *Liquid phase diffusion of the analyte to the electrode interface.* This process is well described in the literature and typically described by the Ilkovic-type or Cottrell-type equations. Many textbooks can be consulted [e.g., 7] for descriptions of this phenomenon.

The next three steps are part of the electrochemical reaction mechanism and are often considered together.

(5) *Adsorption onto the electrode surface.* In CO sensing this step is very rapid.

(6) *Electrochemical reaction.* This includes the electron transfer reaction and it is slower for CO.

(7) *Desorption of the products.* If the product of the reaction does not desorb from the electrode surface, the electrode becomes passivated (poisoned). The sensor can lose its sensitivity.

(8) *Diffusion of the products away from the electrode surface.* This process cleans the working electrode area and returns this area of the sensor to its prior "clean" condition.

Consideration of these last few steps illustrates the importance of the electrochemistry that is occurring in the sensor. If the products are sensor poisons, the sensor lifetime or response may be severely limited.

(9) *Exhaustion of the products.* This process simply cleans the sensor inlet area of products and prepares it for the next analytical task. If the products of the electroreaction are not gaseous, the products may remain in the electrolyte. Of course, if the products are not exhausted, the internal changes in the sensor may cause changes in the response and such effects must be considered.

Any of these steps can be rate limiting, and, therefore, determine the ultimate response characteristics of the amperometric sensor. The parameters that most frequently influence the observed analytical characteristics of a sensor include: sample flow rate, working electrode composition, type and amount of electrolyte, membrane porosity and permeability, and the electrochemical potential of the working electrode. By controlling these parameters, the sensor engineer can often achieve the desired and/or optimum sensor response characteristics for a particular compound in a given situation. Of course, as with any chemical reaction, the electrochemical sensor reaction is typically sensitive to temperature, concentrations of products and reactants, and pressure.

Steps 5, 6, and 7 in the proposed mechanism are especially important because they involve the electrocatalytic phenomena. It is often appropriate to consider steps 5, 6, and 7 as a single, albeit sometimes complex, electrocatalytic event. The response of an electrode may be complicated by electrode poisoning processes and/or autocatalytic reactions (observed as a change of steady-state signal magnitude and response time). However, these problems are well known to electroanalytical chemists and do not detract from the rich possibilities that amperometry offers to the analyst who wishes to design a simple, low-power gas sensor.

The electrochemical sensor's limiting reaction mechanism may change with sensor operating conditions. For example, at low sample rates, the sensor may electro-oxidize all the available analyte and act as a coulometric device being limited only by the supply of analyte [8,9]. Conversely, at high analyte flows, diffusion through the electrolyte may limit response. At low electrocatalytic activity, the rate of reaction at the electrode surface may limit the observed signal current (i.e., the sensor signal).

Before an appropriate model can be developed for a specific sensor, the nature of the rate-limiting step must be determined. The precise mechanism of the electrochemical process can be understood by careful analysis of the transient and steady-state current response and the time dependent concentrations of intermediates and final reaction products in the sensor. A general model of amperometric sensor response may be helpful but complete prediction of response is not yet possible [1] except in a few isolated cases [2-6,10].

A practical amperometric gas sensor design is illustrated in figure 19, and this is only one of many possible geometries. The exposure cap is a mechanical device designed to protect and support the sensor's electrodes, and, often, limit the gas exposure. The working reference and counter electrodes, together with the electrolyte, form the main functional electrochemical components of the sensor. A wick separator is used to insulate the working electrode from reference and counter electrodes and provide for electrolytic contact among the electrodes. The optional hydrophilic wick must be an efficient connection between electrodes. The pressure difference between the inner and the outer parts of the sensor is balanced by a small hole (vent in fig. 19) in the end cap. The pressure must be balanced to prevent electrolyte from leaking through the gas permeable membrane that covers the working electrode. The effect of each element in this electrochemical sensor on the performance characteristics will be discussed in section 3.

2.2 The Limiting Current: Steady-State Sensor Response

The most important parameter in amperometry is the observed current, which is often described by the Cottrell Equation. But there are several different mechanisms that can control the limiting current. This current is most easy to observe in the absence of a background current. The advantage of constant potential amperometric measurements is that faradaic currents are observed at fixed electrode potentials. Under these circumstances, capacitive currents no longer contribute to the overall cell current, and typically much lower detection limits are obtained when compared to other voltammetric methods.

It is usually preferable that a sensor work in a "limiting current region" in which the magnitude of the sensor signal is practically independent of the electrode potential. In theory, the limiting cur-

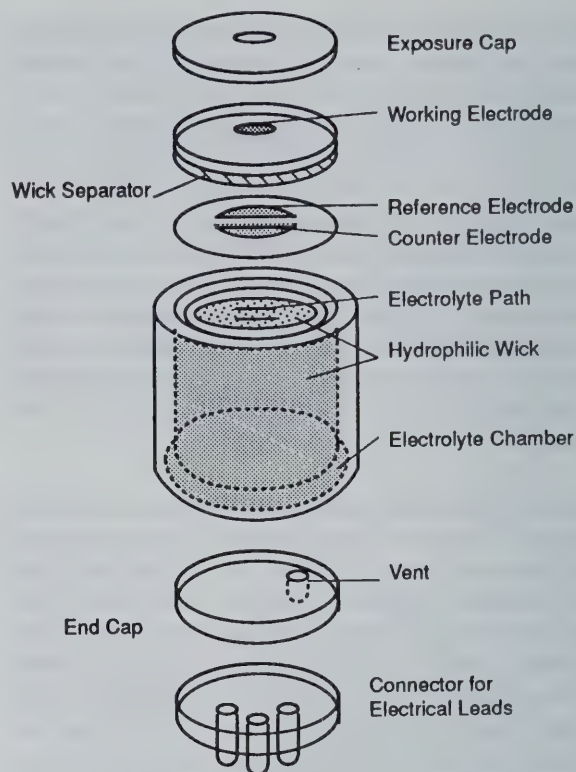


Figure 19. A geometry of a practical amperometric gas sensor.

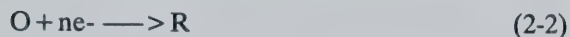
rent region can be achieved, in any case, when the rate-limiting step is a step before electron transfer. This means that any one of the many sensor processes (see prior section, steps 1-9) can control or limit the observed response. In commercial sensor design, a diffusion-limited current is by far the most commonly employed approach. All that one needs to understand the currents produced by amperometric sensors are contained in Fick's Law and Faraday's Law. The combination of these two laws and their application to the appropriate situation (gaseous or liquid phase) for a given sensor will produce the possibility to model the sensor response.

The basic law governing the rate of any diffusion processes is Fick's equation. For one-dimensional processes it can be written as:

$$J_s(x,t) = -D_s[dC_s(x,t)/dx] \quad (2-1)$$

In (2-1) the flux of a substance "s" at a given location "x" at a time "t", written as $J_s(x,t)$, is the net mass transfer rate of "s" in units of amount per unit time per unit area (e.g., mol/sec cm²). Thus, $J_s(x,t)$ represents the number of moles of "s" that pass a given location per second per cm² of area normal to the axis of diffusion.

If O represents the electroactive substance that undergoes reaction to product R, and n is the number of electrons e-,



assuming no other electrode reaction occurs, the current density, i/A is related to the flux of O at the electrode surface ($x=0$), $J_o(0,t)$, by the equation:

$$J_o(0,t) = i/nFA = -D_o[dC_o(x,t)/dt]_{x=0} \quad (2-3)$$

The steady-state limiting rate of a diffusion process, when the concentration at the electrode surface is negligible, can be expressed as:

$$J_{s(lim)} = D_s C_s O / \delta \quad (2-4)$$

where $J_{s(lim)}$ is the limiting flux of "s", δ is the length of the diffusion path, and C is the concentration of "s" before entering the diffusion barrier.

There are several possibilities for realization of the diffusion limited current. First, the rate of electrode reaction may be limited by the rate of diffusion through a membrane or a capillary that is placed somewhere between the gas stream containing the analyte and the catalyst layer of the electrode. The limiting current i_{lim} , (not current density), according to eq (2-4), can be written for any species "s" as:

$$i_{lim} = nFD_s A C_s O / \delta \quad (2-4a)$$

where n is the number of electrons involved in the electrochemical reaction per molecule of the diffusing species, F is the value of the Faraday (96,485 Cmol⁻¹), A is the effective area of the membrane or the cross section of the capillary, and δ is the thickness of the membrane or the length of the capillary. The diffusion constant, D_s , also represents the gaseous diffusion constant for "s" in the medium of the membrane or capillary.

Second, the current can be limited by the rate of diffusion across the diffusion layer in solution. An expression similar to eq (2-4a) can be used to calculate the current. However, in this case, A in eq (2-4a) is the effective surface area of the electrode, D_s is for diffusion of "s" in solution, and δ is the thickness of the diffusion layer in solution at the electrode surface. This is a typical interpretation of Fick's equation in polarography and leads to the well known Ilkovic and Cottrell type equations [7].

There are ways to realize potential independent limiting currents that are not governed by the diffu-

sion process. The current may be limited by transport of the analyte to the sensor from the outside world. In this case, 100% of the electroactive species that enters the gas sensor reacts, and the limiting current is related to the sample in a coulometric way. This type of sensor operation has been used successfully to determine the electrochemical characteristics for several gas-sensor reactions [8,9,11].

If the rates of diffusion processes are much faster than the rate of reaction at the electrode, then the current is controlled by the analyte reaction kinetics at the electrode surface. In this case, an expression for the current will take the form:

$$i = nFKA [P]^p [Q]^q \exp(\alpha n F/R T \beta) \quad (2-5)$$

where K represents the standard rate constant, p and q represent the order of the reaction for reactants P and Q respectively, and α and β are the transfer coefficient and overvoltage of the electrode reactions respectively. This behavior has been observed for an NO₂ gas sensor with a very low surface area electrode [10,12] in acid electrolyte.

Theoretically, the above model has several interesting consequences. If the kinetics of transport of reactant to the electrode surface (up to step 5) are significantly slower than all other steps, the limiting current should be independent of potential. Conversely, a strong potential dependence should be observed for sensors wherein the electrocatalytic reaction limits the observed current, and this "electrode-reaction" limited sensor should produce limiting currents that are independent of external events such as analyte flow rate. These predictions have been recently confirmed for the electrochemical reaction on NO₂ at a gold electrode in acid electrolyte [10]. Finally, we conclude that a comprehensive model to explain the performance of the amperometric gas sensor under all conditions may be a complex combination of Fick's laws and Faraday's law.

2.3 Conclusions

Several major conclusions can be made from the above brief introduction to amperometric sensors.

- (1) All of the explanations of the relationships among current, potential, time, and chemical reactions are made by combining Fick's law, Faraday's Law, the Nernst Equation, and some expression for

the *Rate* of arrival or *Rate* of reaction of analyte at the electrode/electrolyte interface. This is an important observation and significantly simplifies this apparently diverse field.

(2) The rate of mass transport can be well defined by choice of sensor geometry and under certain conditions in specific cells, the electrochemistry of the gas sensor can be studied. Conversely, when the chemistry is known, mass transport can be studied by using the amperometric gas sensor.

(3) The equilibrium constant as well as the electron transfer rates for an electrochemical process will vary with potential. This allows study of thermodynamic properties and kinetic parameters of gas reactions by use of amperometric gas sensor.

These three conclusions provide the basic ideas and methods that are useful in studying, improving, and applying amperometric gas sensors.

3. Performance Characteristics

The amperometric gas sensor is an electrochemical cell. Its detection of gases and vapors is performed either by an oxidation or reduction of the compound at an electrocatalytic surface that is in contact with an electrolyte, which may be a liquid, solid, or gel. Some sensors operate by reaction of the electrode itself. These systems are also important and, as one might surmise, can have very different analytical characteristics. The myriad of amperometric sensor possibilities are only limited by the imagination and skill of the scientist or engineer. The analytical performance of the sensor is closely related to the selection and preparation of materials and the geometry of the sensor. This can be appreciated by consideration of a typical sensor specification for an environmental application (see table 4). The ability to achieve a practical sensor with such operating characteristics is the analytical challenge.

3.1 Major Components of Amperometric Sensors

The physical structure of an amperometric gas sensor may be divided into the following five major components:

- Potentiostat (electronics)
- Electrodes (electrocatalysts)
- Membranes (diffusion barriers)

- Electrolytes
- Gas inlet and outlet (sampling system).

Table 4. Example of sensor specifications*

Parameter	Specification/comment
Lifetime	1 year between maintenance cycles.
Rage	0.1–10X level of interest, linear $\pm 2\%$.
Sensitivity	0.1 of level of interest.
Precision/accuracy	$\pm 2\%$; $\pm 5\%$ over sampling period (8 hrs).
Response time	90% in < 30 sec.; decay = same. initial response rapid.
Flowrate	pump or diffusion, no span change.
Span/output	compatible with data capture/display < 10% drift 0–40 °C or 0–100% RH.
Noise	min S/N = 50:1 @ level of interest.
Background/zero drift	within accuracy/precision specification.
Stabilization time	< 15 minutes.
Selectivity	no response to common gases; specific to compound or class of interest; specify interferences, levels, ratios.
Temperature	– 20 to + 40 °C (see zero/span drift).
Relative humidity	0–100% (1–99%, 5–95% OK).
Mechanical	small size, wt., rugged, with connector. self-contained; rugged, no consumable.
Power	battery or AC; requires only microamps.

* The acceptable specifications are totally dependent upon the series of interest and the application.

3.1.1 Potentiostat and Associated Electronics

A potentiostat is usually used to provide a fixed potential for the working electrode relative to a reference electrode, which is placed in the same electrolyte. In a three-electrode system, the potentiostat is employed to energize and control the electrochemical cell as well as to convert the sensor's current signal to a voltage and amplify it. Early potentiostat designs suffered from poor control of the potential but with the advent of the high-quality operation amplifier, these problems are largely things of the past. Modern electronics and computer control have given the experimentalist an improvement in the diagnostic and analytical

power of electroanalytical systems. The simple circuit shown in figure 20 is often embellished in commercial potentiostats by gain and offset adjustments as well as noise suppression circuitry and computer control, but is essentially the same. A computer-controlled bias voltage, V_0 , allows time-dependent variations to perform the wide variety of voltametric, $I(t)$ vs $E(t)$, experiments. A discussion of the potentiostat and its interaction with the electrochemical cell can be found in many textbooks [7]. But it is useful to point out here that the electronics and sensor are in intimate contact and must be considered as a system to achieve optimum sensor performance. The design of instrumentation [13] can be complicated by idiosyncrasies of either the sensor or the electronics.

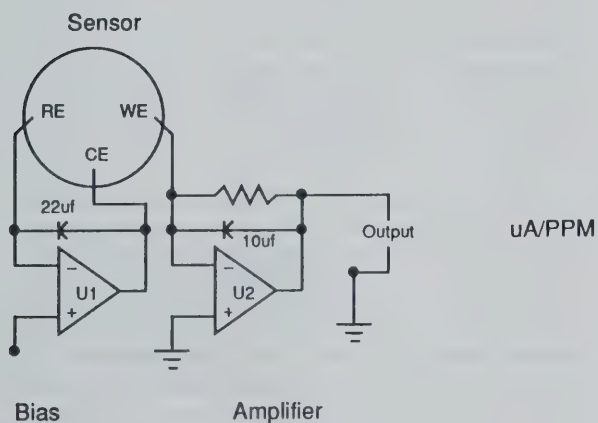


Figure 20. A simple circuit for illustrating the operation and control of an electrochemical sensor and the display of signals.

It should be realized that the bias voltage (potential between WE and RE) has a direct effect on the selectivity of the sensor. For instance, a bias voltage of +1.30 V anodic to the SHE (standard hydrogen electrode) was used with a gold electrode to perform NO oxidation, while a voltage of 0.75 V, versus the same reference electrode is used for NO₂ reduction in the same sensor [14]. The NO oxidation is not observed at 0.75 V, so that the selective detection of NO₂ in the presence of NO is possible with a simple amperometric sensor.

Some of the newer variants of amperometry do involve pulsing the electrode potential in and out of the potential region where the analyte is electroactive to enhance the sensitivity and selectivity [15]. It has even been reported that cyclic voltammetry can be used for gas detection, but the success of this approach is not well documented [16].

3.1.2 Electrodes/electrocatalyst To be useful as a chemical sensor, the electrode must be capable of producing an electrical signal that is related to the concentration of the analyte. In an amperometric sensor, the analytical signal is realized by the generation of a current that arises directly from the oxidation or reduction of the analyte. The sensor can contain two or three electrodes. Two-electrode systems for O₂ and CO are common and in these sensors, the auxiliary electrode serves as both counter and reference electrodes.

The three-electrode configuration allows for precise operation even with micro-electrodes. The reference electrode is used to maintain the working electrode at a known potential; it must, therefore, be physically and chemically stable in the electrolyte. Typically, the working electrode is a noble metal gas-diffusing electrode which is capable of keeping the electrolyte in the cell and yet is porous enough to allow the gas phase to diffuse to the interface of electrode and electrolyte. Since the reference electrode should carry the lowest possible current in order to maintain a constant potential, the counter electrode completes the electrochemical cell by performing the half cell reaction. The nature of this reaction must be in opposition to the working electrode reaction in order to minimize net chemical changes in the sensor.

The invention of the Teflon-bonded noble metal catalyzed gas-diffusion electrode was a breakthrough and led to the development of many modern amperometric gas sensors. The introduction of a Pt/air reference electrode significantly simplified sensor design and produced practical applications of the technology. However, the future of electroanalytical chemistry now surely lies in the development of new useful working electrodes and electrochemical systems. The design, preparation, characterization and application of chemically modified electrodes is a very important aspect in modern sensor development.

3.1.3 Membranes/Diffusion Barriers There are six advantages to covering the electrodes of an amperometric gas sensor with a membrane that is permeable only to the analyte [15] or that has special functional properties. Poisoning of the electrodes by electroactive or surface-active species is reduced. The selectivity is enhanced if extraneous electroactive species that would otherwise undergo electron transfer at electrode, can be excluded. The composition of the electrolyte that occupies the space between the membrane and the electrodes remains substantially constant. The membrane forms a physical barrier to the spread of the

diffusion layer into the bulk solution. The membrane structure is often chosen to be hydrophobic to reduce or eliminate aqueous electrolyte loss to the outside world and increase sensor lifetime. A diffusion barrier can also be used to control and reduce the sensor response to changes in such environmental conditions as sample flowrate and temperature.

Several types of porous and gas-permeable membranes exist and can be made of polymeric or inorganic materials. Typical membranes include very thin (0.5 mil) solid Teflon film, microporous Teflon film (Zitex or Gore-Tex), porous polypropylene films (Celguard), or silicone membranes. Silicones are preferentially permeable to organic vapors [17]. Issues concerning the choice of membrane include the permeability to the analyte of interest as well as the thickness and durability of the membrane. Slow and small responses may be observed if the rate of permeation of the analyte through the membrane is restricted. Of course, it is often useful in application engineering to choose a membrane that optimizes selectivity, or durability to extreme chemical or physical stresses, at the cost of response time or sensitivity.

3.1.4 Electrolytes The functions of the electrolyte are to efficiently carry the ionic current, solubilize the analyte, support both counter and working electrode reactions, and form a stable reference potential with the reference electrode. Exemplary materials for sensor electrolytes are given in table 5. The major criteria for an electrolyte include:

- Electrochemical inertness
- Ionic conductivity
- Chemical inertness
- Temperature stability
- Cost
- Toxicity
- Flammability
- Viscosity (for a liquid electrolyte).

Electrolytes can enhance the selectivity of the sensor if the analyte is selectively soluble or if the electrolyte reacts selectively with the analyte to form a chemical species that can be detected more easily. On the contrary, if complex formation occurs between the analyte and the electrolyte, electro-reaction can be impeded. For example, when N_2H_4 is electro-oxidized in acid solution:



but in basic solution:



In acid solution, the sensor signal is not repeatable (oxidation is impeded), while the signal is very well behaved in basic electrolyte [18]. The behavior of molecular hydrazine is quite different from that of the complexed protonated species, and the sensor performance is also quite different because of the choice of the electrolyte.

Table 5. Examples of electrolytes

Aqueous electrolytes	<i>Acid:</i> sulfuric, perchloric, phosphoric HCl, HNO ₃ , Acetic acid, <i>Base:</i> NaOH, CsOH, LiOH, KOH <i>Neutral and salt:</i> KCl, LiCl, LiBr, NaHCO ₃ , acetate.
Non-aqueous	<i>Solvents:</i> Propylene carbonate, DMSO, DMF, Acetonitrile, ethylene glycol, Dichloromethane, propylene glycol, pyridine, THF. <i>Electrolytes:</i> perchlorate salts, LiCl, tetraalkylammonium tetrafluoroborate and hexafluorophosphate salts, tetraethylammonium tetrafluoroborate, trifluoromethanesulfonate.
Solid and polymers	Nafion, PEO*LiX, PVA/H ₃ PO ₄ , PVA/CsOH, and Inorganic Solids, ZrO ₂ .
Other	<i>Mixtures:</i> Water-glycol with salts, etc.

Another example of electrolyte effects on sensor performance can be illustrated by study of the electrochemical reduction of NO₂ in acidic and basic electrolytes. The reduction signal for NO₂ on gold electrodes persists to more anodic potentials in basic electrolyte than in an acidic medium. This is consistent with the observation that the gold surface has a lower oxide coverage in base than in acid at the same potential [19,20]. In this example, the pH of the electrolyte has a profound effect on the sensor performance. The commercial NO₂ sensor, however, most often utilizes an acid electrolyte because basic electrolytes can form carbonates upon exposure to the air which makes them unstable.

Nafion is the solid polymer most often described in the sensor literature as an electrolyte for a sensor or as a membrane. Nafion is a copolymer of PTFE (Teflon or polytetrafluoroethylene) and polysulfonylfluoride vinyl ether containing sulfonic acid groups. The sulfonic acid groups are chemically bound to the perfluorocarbon backbone. The

polymer has much of the mechanical and chemical inertness properties of Teflon-like polymers. The proton within this structure becomes mobile when the polymer is wet. In a typical sensor design, a Nafion film is used both as the electrolyte and as a support for the electrode structure. The electrodes are physically attached to the Nafion using heat or chemical bonding techniques. Nafion sensors have been used with a distilled water filling for several gas-sensing applications [12,14,21–23] and offer the advantages of a rugged electrolyte, and no risk from spilling corrosive electrolytes. A disadvantage of Nafion is that the water will freeze at about 0 °C, whereas aqueous acid or base electrolytes can be used at extremely cold temperatures.

3.1.5 Sample Gas Access In most gas sensors, a sampling system, usually a pump or diffusion tube, is used to transport the reactive gas to the working electrode. A major problem often exists in obtaining a gaseous sample that is representative of the sampled environment so that the observed sensor current has analytical significance. Ozone, which reacts with many materials, is an example. If the analyte gas is corrosive, the sensor must be made of chemically resistant materials like Teflon or polypropylene. Frequently, the sample must be filtered to remove particulate and condensable materials as well as some interfering or reactive compounds. A selective chemical filter is often used to enhance the selectivity of the amperometric sensor and thereby improve the sensor performance in field applications. For example, a combination of potassium permanganate on activated alumina can remove gases like SO₂, H₂S, NO, and NO₂ from the air but allow CO to pass through to the detector. Such filters can greatly enhance the selectivity of a sensor, although their suitability is very dependent on the application.

The sensor component parts are typically polypropylene or Teflon to provide for chemical resistance in industrial applications. The electrical contacts are gold plated so that the sensor can be used in corrosive atmospheres for extended periods of time. The gas inlet and outlet system may be designed such that a small pump can easily push or pull a gaseous sample past the cell membrane. The gas inlet may be a simple hole to allow transport of the sample to the working electrode area by diffusion or convection. The geometry of the inlet and outlet is extremely important and can significantly influence sensor performance.

A sensor, wherein the supply of analyte is limited by the inlet geometry, will assume the kinetic response properties of the inlet and not those of the

electrochemical reaction within the sensor. This can be advantageous if a gaseous diffusion limit for the analyte is approached, because the temperature dependence of this signal will then vary with $T^{1/2}$ rather than the typical exponential dependence of a chemical reaction. The molecular theory of the property of diffusion can be approximated by:

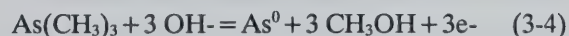
$$D = 3/8(R T/\pi M)^{1/2} (1/\sigma^2 n) \quad (3-3)$$

as discussed in Kauzmann [24]. In eq (3-3), n is the number of moles, M is the molecular weight, R is the gas constant, σ is the effective (collision) diameter of the analyte molecules, and T is the temperature. This equation predicts the sensor signal will be dependent upon $T^{1/2}$ if gaseous diffusion limits the current produced by the sensor. A compromise must be made with lower sensitivity in return for a low temperature coefficient of the signal. This compromise is often acceptable, since modern electronics can easily amplify small signals with precision.

3.2 Analytical Characteristics of Amperometric Sensors

3.2.1 Sensitivity The sensitivity of electrochemical sensors is a function of many variables, such as mass transport of the compound within the detection system, electrocatalytic activity (the catalyst material, its physical form, and the electrochemical potential at which it is operated), Faradaic equivalents per mole transferred during reaction, analyte solubility and mobility in the electrolyte, the physical geometry of the sensor, and the means of sample access (including semipermeable membranes or filters). The optimization of these parameters leads to the maximum signal-to-noise ratio and other analytical specifications for a given amperometric sensor system.

The signal from amperometric sensors is often quite large by electronic standards. For example, reaction of 1.0 cc of 0.3 ppb TMA (trimethylarsine) according to the equation,



will produce 3.9×10^{-9} coulombs. If only 10% efficiency can be obtained during a second analysis time, a current of 0.39 nA will be observed. Indeed, ppb and even ppt levels should be easily detected by modern electronics. The practical sensitivity limit for most sensors today is not the signal size. Rather, it is the relatively large magnitude of the

background and noise currents. The possible causes of background current in amperometric gas sensors can include:

- Impurities in the electrodes or the electrolyte such as traces of dissolved oxygen or metals (these may also affect the selectivity of the sensor),
- Slow oxidation or reduction of solvent at the working electrode, such as the slow evolution of hydrogen or oxygen from an aqueous solution,
- Corrosion of the electrode, such as the slow growth of the oxide layer on the surface of a noble or passivated metal electrode catalyst in the anodic potential range,
- Impurities that are electroactive that are in the sampled gaseous atmosphere as background materials at low levels, and
- Diffusion of the reactant or reaction products from the counter electrode, such as the diffusion of dissolved peroxide toward the sensing electrode from an oxygen-reducing counter electrode.

The practical sensitivity of amperometric sensors has been in the ppm range for many years and, for modern sensors, has been extended to ppb levels for selected analytes like NO₂ [10–12,25–27], Cl₂ [28], and H₂S [29], to name only a few. It should be remembered that achieving sensitivity for its own sake is an academic challenge worthy of research. Practical sensor sensitivity is that which is sufficient for the application and often can be achieved today with amperometric gas sensors.

3.2.2 Selectivity Selectivity in sensors is an extremely important and complex issue. A complete discussion is well beyond this summary. Fundamentally, there are only two variables controlling selectivity. It must be based upon the thermodynamic or kinetic properties of the system. The prior example of NO₂ sensing in the presence of NO at cathodic potentials is an example of selectivity based on thermodynamics. Generally, the selection of electrocatalyst and electrode potential offers the first opportunity for selectivity. Then, electrolytes, operating methods, or sampling procedures (for example, incorporating selective filters or membranes) are chosen. The ultimate sensor design and resulting selectivity depends upon the requirements of the application and the creativity of the individual who designs the sensor.

Selectivity is often the weak point of some chemical sensors and significantly limits its application to solve practical problems. Many approaches with the specific goal of increasing selectivity have been attempted, however, no comprehensive theory or

formula is available today to achieve selectivity. Empirical approaches are reported most frequently. One popular method for enhancing sensor selectivity is to use chemical filter to remove interfering gases [30]. A newer method is to use a sensor array. An array consisting of four electrochemical sensors and two hot wire filaments has been evaluated [30–32]. The ratios of sensitivities among different sensors provides qualitative analytical information not available from a single sensor. In other words, the selectivity of an array can be much better than that of its individual sensors.

3.2.3 Response Time In safety applications, the speed of response is important, and it can be controlled to a large extent in amperometric sensors. The response time of the gas sensor has been treated empirically for the most part. For instance, the response of the diffusion electrode to carbon monoxide [11] followed a first-order law according to this equation:

$$i = i_s (1 - e^{-\alpha t}) \quad (3-5)$$

but for hydrogen sulphide [25], it followed a second-order rate law:

$$di/dt = k (i_s - i)^2 \quad (3-6)$$

where i is the current (signal) at any time t , i_s is the current at the steady-state, is the sensor response-time constant, and k is the rate constant.

The response time of a potentiostatically controlled amperometric sensor is, however, highly dependent upon the impedance between the reference electrode and the working electrode, i.e., the solution resistance, and specific instances have been described [17] in some detail. It is also possible that the response time of a sensor is limited by the mass transport of analyte to the reaction cell. In this case one can slow the response by placing a very thick membrane in front of the cell. We are usually interested in the reverse situation-fast response, and this will be achieved by improving mass transport into the system. In other words, small structures that are very permeable and open to the analyte will reach steady state most rapidly. This leads to response controlled by the electrode processes. Observed responses of micro-amperometric sensors in the millisecond range have confirmed these statements [23].

3.2.4 Noise Considerations Recently, there have been some speculations on noise generation of electrochemical sensors. Lankelma and Poppe [34] suggested that noise should be proportional to

electrode area. Weisshaar et al. [35] concluded that the Kel-F-graphite composite electrode can minimize flow-associated noise since the electrode is made up of many small electrodes at which steady-state diffusion layers exist. A detailed discussion of noise and signal-to-noise ratio in amperometric sensors was presented by Morgan and Weber [36]. Temperature variations are also a source of noise [15]. The use of a combination of active and passive thermal shielding has been shown to decrease the amount of low-frequency noise power by an order of magnitude [36]. The well-understood techniques of grounding and shielding that can prevent capacitive pick-up of electrical radiation are always directly applicable to electrochemical sensors.

Careful sensor design with respect to electrical and thermal shielding is essential, as is the use of low-noise electronic components in the measuring circuits. To a large extent, noise considerations can and should be taken into account in the design stage of the sensor by the sensor engineer. This process is not different from the requirements of any other analytical instrument.

4. Applications Today

As we have learned, amperometric gas sensors are based on measurements of steady-state currents that are proportional to the concentrations of the analytes. By using the principle that the diffusion-limited current is proportional to the partial pressure of alcohol in the breath, the alcohol sensor was developed [21,37]. This sensor is now used by police officers at the scene of traffic violations either as a screening instrument or as an evidential device. Stetter et al. developed an amperometric sensor for the determination of hydrazine and methyl- and 1,1-dimethylhydrazine [38]. This sensor can provide fast, accurate, sensitive and real-time determination but has a limited lifetime. A similar approach has been used for the determination of the oxides of nitrogen [39] and for hydrogen sulphide [29]. The hydrazine and NO₂ sensors were developed in conjunction with NASA's Space Shuttle Program as part of the hazardous-vapor-detection system [40] and are presently considered relatively old technology. For detection of carbon monoxide in the range 0 to 250 ppm, a portable battery-operated instrument was developed for use in coal mines to further protect the health and safety of the mine workers [41]. Another application for the coal mine industry is the detection and

measurement of methane by using amperometric sensors [42–43].

In clinic chemistry, an O₂ and CO₂ gas sensor for the determination of the partial pressure of these gases in the blood and the determination of the blood acid-base status [44] has found important application. In biochemistry, amperometric sensors (although not gas sensors) have been applied to the determination of proteins, root death, total iron, and the concentration of NO₃⁻ [45]. It has been reported that the glucose oxidase enzyme can be immobilized at the surface of the oxygen electrode [and the oxygen deficiency at the working electrode due to the enzymatic oxidation for in vivo and ex vivo monitoring of glucose [46], lactate [56–58], xanthine [59], ascorbic acid [60], microbial growth [61–62], dopamine [63], and salicylate [64–65].] The utilization of amperometric sensors of all kinds for the determination of environmental pollutants has been reviewed recently by Smart [66]. The application of the amperometric gas sensor is extremely diverse and only limited by the imagination of the scientists and engineers who design and use them.

Amperometric gas sensors have been used to design instruments for chemical detection and a wide variety of both portable and fixed-site toxic-gas monitors exist today. These instruments have found applications in monitoring industrial atmospheres, medical gases, compressed-air lines, process streams, and pipeline gas. Gas chromatographic instrumentation, ventilation controllers, energy-management systems, environmental-pollutant-control equipment, chemical-research instrumentation, and analytical laboratory gas analyzers also utilize amperometric gas sensors. An interesting comparison of the many types and applications of the amperometric gas sensor can be obtained by examination of the technology summarized in table 6. This table provides a quick overview of some of the analytes that have been addressed by the amperometric approach.

5. Technology Gaps

Although electrochemical gas sensors that utilize such liquid electrolytes as H₂SO₄ HClO₄ are available to detect inorganic gases, (e.g., O₂, CO, Cl₂, H₂S) at room temperature [67–68], they often have time-related problems such as leakage and corrosion, and their signals are often observed to decay as electrolytes evaporate or become contaminated. Such instabilities often can be controlled by a judi-

Table 6. Comparison of electrochemical sensors

First author	Sample	Range	Electrode	Electrolyte	Proposed reaction	Ref.
Bay, H. W.	C ₂ H ₅ OH	ppm	Pt	H ₂ SO ₄	C ₂ H ₅ OH + (O) → C ₂ H ₅ OOH	[29]
Cook, R. L.	CO ₂	ppm	WE-Cu, CE-Pt	Nafion117	CO ₂ + [R] → C ₂ H ₄ , C ₂ H ₆ , CH ₄	[74]
Bay, H. W.	CO	ppm	Pt	H ₂ SO ₄	CO + H ₂ O → CO ₂ + 2H + 2e	[7]
Sedlak, J. M.	H ₂ S	sub-ppm	Pt	Nafion	H ₂ S + 2O ₂ → H ₂ SO ₄	[24]
Sedlak, J. M.	NO, NO ₂ , CO	sub-ppm	Au	H ₂ SO ₄	NO + 2H ₂ O → NO ₃ + 4H + 3e	[20]
Blurton, K. F.	CO	ppm	Pt, PTFE-bonded	H ₂ SO ₄	Pt + [O] → PtO	[75]
Sedlak, J. M.	NO, NO ₂	ppm	Au		NO ₂ + 2H + 2e → NO + H ₂ O	[31]
LaConti, A. B.	H ₂	ppm	Pt	H ₂ SO ₄	H ₂ → 2H + 2e	[76]
Stetter, J. R.	Hydrazine	ppm	Au or Pt		N ₂ H ₄ + 4OH → N ₂ + 4H ₂ O + 4e	[30]
Stetter, J. R.	Hydrazine	ppm	Au, PTFE-bonded	H ₂ SO ₄ , KOH	N ₂ H ₄ + 4OH → N ₂ + 4H ₂ O + 4e	[16]
Blurton, K. F.	H ₂ S	ppb	Pt, PTFE-bonded	H ₂ SO ₄		[77]
LaConti, A. B.	CO, NO, NO ₂	ppm	Pt, PTFE-bonded	H ₂ SO ₄	CO + H ₂ O → CO ₂ + 2H + 2e	[13]
Tantram, A. D. S.	O ₂		Pt-Ag mixer	KOH		[78]
Becker, W. J.	SO ₂ , NO ₂ , Cl ₂ , etc.	ppm	Pt	Gel electrolyte		[79]
LaConti, A. B.	H ₂ S, CO, CH ₄ N ₂			H ₂ SO ₄ , H ₃ PO ₄		[80]
Mark, Jr., H. B.			Pt or Au			[81]
Gifford, P. R.	Hg, I ⁻ , IO ₃ ⁻	ppb	Au	H ₂ SO ₄		[82]
Hahn, C. E. W.	CO ₂ , O ₂	ppm	Pt	NaCl or KCl	O ₂ + 2H ₂ O + 4e → 4OH	[3]
Hahn, C. E. W.	halothane, N ₂ O	%	Ag	KOH, KCl		[83]
Van Rooijen, H. W.			Au or Pt			[84]
Stetter, J. R.	CO	ppm	Pt			[85]
Otagawa, T.	CH ₄	ppm	Pt, PTFE-bonded	NaClO ₄	CH ₄ (g) + H ₂ O(l) → CH ₃ OH(aq) + 2H + 2e	[34]
Otagawa, T.	CH ₄	ppm	Pt, PTFE-bonded	NaClO ₄	CH ₄ (g) + H ₂ O(l) → CH ₃ OH(aq) + 2H + 2e	[35]
Stetter, J. R.	CO, etc.	ppm	Pt, PTFE-bonded	H ₂ SO ₄	A + O ₂ → CO + B	[86]
Otagawa, T.	CO, etc.	ppm	Pt, PTFE-bonded	H ₂ SO ₄	A + O ₂ → CO + B	[87]
Cook, R. L.	CO ₂	ppm	Cu	KHCO ₃	4Cu + CO ₂ → 2Cu ₂ O + C	[88]
Cook, R. L.	CO ₂	ppm	Cu	KHCO ₃	4Cu + CO ₂ → 2Cu ₂ O + C	[89]
Moseley, P. T.	H ₂ , CO, NH ₃ , etc.	ppm	Pt or Au	water		[90]
Cook, R. L.	CO ₂	ppm	Cu	KHCO ₃	CO ₂ → CH ₄ , C ₂ H ₄ , C ₂ H ₆	[91]
Penrose, W. R.	NO ₂	ppb	Pt	H ₂ SO ₄		[21]
Evans, J.	CO ₂	ppm	Pt	KCl	CO ₂ + H ₂ O → 2H + CO ₃	[92]
Enea, O.	C ₂ H ₅ OH	ppm	Au, Nifion	NaOH		[93]
Fabjan, C.	O ₃	ppm	Pt	H ₂ SO ₄	O ₃ + 2H + 2e → H ₂ O + O ₂	[94]
Albery, W. J.	CO ₂ , O ₂	ppm	Hg	DMSO		[95]
Belanger, G.	SO ₂	ppm	PbO ₂ on Au	H ₂ SO ₄ & HClO ₄	SO ₂ + 2H ₂ O → SO ₄ + 4H + 2e	[96]
Bergman, I.	CO	ppm	Au, Ag, Pt	H ₂ SO ₄		[97]
Gifford, P. R.	As, Sn, Sb	ppb	Au GPE	H ₂ SO ₄	As(III) + NaBH ₄ → AsH ₃ + other products	[98]
Beran, P.	HCN	ppb	Au GPE	H ₂ SO ₄	I ₂ + HCN → H + I + ICN	[99]

cious choice of the electrolyte and the operating conditions of the system. For instance, solid electrolytes may be used in place of liquid. It has been reported [69–70] that solid electrolyte sensors using stabilized zirconia can detect reducible gases in ambient atmospheres by making use of an anomalous EMF, which is unusually larger than is predicted from the Nernst equation. Whether this sensor is amperometric or potentiometric may be debated. Another example is to utilize a ferrocene-modified polysiloxane base on glycolate oxidase and polymeric electron transfer mediators as an amperometric biosensor [71].

Other problems in amperometric gas sensors with liquid electrolytes are short lifetime, relatively large size, and high cost (\$25–\$500). The sensors are not as inexpensive as tin-oxide-based solid-state gas-detection device (\$2–\$200). Sensitivity requires improvement for indoor air applications where concentrations are typically in the ppb and

ppt range. Improvements on sensitivity may also lead to other applications wherein low concentrations are important, such as in detection of concealed contraband like bombs, incendiary devices, and drugs. Selectivity is a most frequent problem in real-world applications. Improvements in selectivity can often lead to improvements in sensitivity since unwanted interfering signals disappear. This often results in sensors with lower background currents and hence, improved S/N. Medical and process-control situations demand more rugged and reliable sensors.

In summary, we have cited improvements in almost every sensor performance category as a technology gap. This may seem a trivial observation but the opportunity herein may also be significant. Electrochemical gas sensors have excellent logistical properties: they are very portable, and they require low power. The current requirement for sensors is often for ultrahigh sensitivity, for detec-

tion of trace substances such as impurities, metabolites, pollutants, explosives, or drugs. The possibility to achieve this detection in a low-cost design is significant using the amperometric sensor approach. Further, the combination of electroanalytical chemistry and microfabrication technology [22,72] can offer the potential for very low cost as well as uniformity of performance.

6. Opportunities and Concluding Remarks

The continuing publications concerning chemical sensors [73] and—in particular—amperometric sensors [15,74,75] underscore the continuing importance of this electroanalytical approach in the fabrication of practical gas sensors. There are over 1500 references cited in a single analytical chemistry review article under the general entry of “chemical sensors” for the period between January 1985 and December 1987 alone [76]. The field of sensors is interdisciplinary, and future advances are likely to occur from unique combinations of advanced technologies in other fields with the known way to utilize electrochemistry in gas sensors.

Microelectronics has produced a variety of physical and chemical sensors, but only a very few can be considered amperometric [74], and these structures usually do not contain the electrolyte. Most microsensors are potentiometric, and more common amperometric gas sensors (e.g., CO, H₂S, NO, NO₂, etc.) have not yet been microfabricated. It is believed that microelectronic fabrication techniques are well suited for the production of amperometric gas sensors. The need for a geometrically well-defined sensing electrode surface area is well recognized. Photolithographic proceeding technique can be used to produce identical, highly uniform and well-defined electrode structures. Thin- and thick-film metallization as well as sputtering techniques can be applied to produce the metallic-electrode elements. Some work has been performed in combining microsensors and microfabrication [23]. Chemical- and plasma-etching techniques can be used to delineate geometrically well-defined electrode surfaces. These microelectronic fabrication techniques provide a fruitful way to prepare sensors with specific geometries. These techniques have only begun to be explored at this time and should aid researchers who need to characterize and understand the sensor system. They should also result in some exciting practical sensor designs.

Sensor arrays are not well developed, but they hold promise to improve sensor selectivity vastly. New electrolyte materials must be found so that integrated sensor structures can be built. A new class of polymers is emerging from this research that react to both chemical and electronic stimulus. Modulation of the sensor signal and the study of time-dependent sensor signals [77] will result in new sensor systems with more information content and enhanced analytical capabilities. This is an exciting time for sensor research and, in the near future, amperometric sensors will be more sensitive, more selective, and smaller; they will possess a longer lifetime and be less expensive. We cannot conclude without a mention of the numerous works of our colleagues in liquid-phase amperometric detection. This classical branch of electroanalytical chemistry finds direct application in HPLC detectors and the many classical electroanalytical methods. Much of the gas-sensor work depends upon the knowledge gained in this type of research. Also, readers should be aware of the large and rapidly growing body of literature concerning potentiometric sensors and other types of electrochemical sensors as well as microsensors of all types for chemical detection. Often, advances in other microsensor technology (e.g., the potentiostat on a chip [78–79]) will offer the possibility of substantial advances in applications and design of amperometric sensor. The challenge posed by our modern society for chemical sensors is great, but the possibility for amperometric gas sensors to make a substantial contribution is also significant.

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Opportunities with Acoustic Sensors

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Acoustic sensors employ vibrating disks or planar structures, driven piezoelectrically, to measure physical, chemical, and biochemical quantities. A variety of wave types may be used: bulk waves that move an entire crystalline solid, surface waves that move just a thin layer at the surface of a slab, and plate waves that either propagate along a thin membrane or progress by bouncing between top and bottom surfaces of a crystalline plate.

Acoustic sensors operate in several different ways. In *gravimetric* sensing, one detects the mass increase caused when metallic, vapor, gas, or biochemical molecules become attached to the sensing surface. With *stiffness* sensing, one detects variations of acoustic wave speed caused by changes of the elasticity

of a substance on the sensor, such as a fluid that is polymerizing. Finally, by monitoring the dissipation of acoustic energy, one can determine mechanical properties, such as the viscosity of a fluid in contact with the sensor.

The past decade has seen substantial research activity in this area, including development of designs that employ microfabrication for low cost and small size. Possible commercial applications include monitoring pollutants, determining viscosity of lubricants, and performing biomedical tests.

Key words: acoustic; biosensor; chemical sensor; delay line; density; gravimetric; pressure; resonator; ultrasonic; vapor; viscosity.

1. Introduction

At the outset, you might wonder why one should consider using acoustical devices such as sensors to detect vapors, or biomolecules, or the viscosity of a fluid. Even if you admit that there might be ways to do this—which we will describe below—why use acoustic sensors for these purposes? There are three different answers to this three-part question. Acoustical vapor sensing is viable because it is so extremely sensitive. Detecting biomolecules with acoustic sensors can also be quite sensitive, and one can design a sensor that operates well in the fluid that contains the molecules, eliminating the need for the separation and drying steps that could alter molecular structure. Finally, because sound waves involve mechanical motion, they are well suited to measuring a mechanical property such as viscosity.

The first acoustic vapor and gas sensors employed vibrating piezoelectric quartz-crystal plates that were originally made for use as the frequency determining elements of electronic oscillators (fig. 21a). The addition of a special coating to absorb vapor or gas molecules formed a sensor whose resonant frequency depended on how many molecules were absorbed. More recently, other sensor configurations have been devised, including ones that use ultrasonic waves traveling along the surface of a solid (fig. 21b), in a very thin membrane (fig. 21c), or in a thick plate (fig. 21d).

To show how these acoustic sensors are useful, we first examine what sorts of acoustic waves can propagate in solids. Then we consider how to generate and detect these waves. Next we discuss how the acoustic waves can be used for sensing and ex-

amine the sensitivities that are achievable. Materials and fabrication methods employed to make acoustic sensors are discussed, and we conclude with some applications. Additional sections list references and tell you where to obtain more information on the subject. The treatment here is nonmathematical, since the cited references fully develop the analytical aspects of the subject.

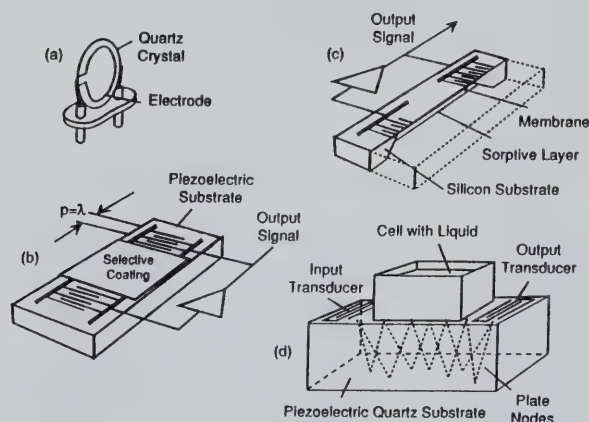


Figure 21. Ultrasonic chemical vapor and gas sensors (see text for details). Credits: (a) After figure 16 in Lu [1]; (b) After figure 1 in Wohltjen et al. [2]; (d) From Ricco and Martin [3].

2. Elastic Waves in Solids

The elastic waves that can exist in solids are illustrated schematically [4] in figure 22. In an unbounded solid, only bulk longitudinal and transverse waves can propagate. The longitudinal wave (fig. 22a) is like a pressure wave in air: the particles of the solid move in the direction of travel, indicated by the heavy arrow. The transverse wave (fig. 22b) is somewhat like the wave you can launch on a taut rope by shaking its end up and down. The longitudinal wave is also known as a compressional wave, and the transverse wave is also called a shear wave.

A surface acoustic wave (SAW) can propagate along the surface of a semi-infinite solid, which has a single boundary (fig. 22c). At all practical frequencies, the speed of the SAW is independent of frequency, as is also the case with bulk waves (typical velocities are shown at the right side of fig. 22). As the sketch of figure 22c suggests, the particle motion of a SAW is largest near the surface and decreases to nearly zero at a depth of about one wavelength. As an example, from the formula for the wavelength (wavelength=velocity/frequency), we find that a 50-MHz SAW propagating on the

surface of lithium niobate, whose SAW velocity is about 3700 m/s, will have a wavelength of 74 μm , or 0.0029 inch. Computer analysis shows that the SAW velocity in any solid is slightly smaller than the speed of transverse bulk waves propagating in the same direction in an unbounded sample of that material.

When a solid is bounded on two surfaces to form a plate, the stresses on both surfaces must vanish. Analysis then yields a doubly infinite set of modes known as Lamb waves, after their discoverer, Sir Horace Lamb. Their velocities depend on the material of the plate and the ratio of plate thickness to wavelength. These plate modes may be divided into symmetric and antisymmetric sets (fig. 22d) according to the symmetry of the motion relative to the center plane of the plate. In plates whose thickness is much smaller than the wavelength, the velocity of the lowest-order antisymmetric mode (a flexural mode denoted A_0) can reach very small values, as low as tens or hundreds of meters per second. Figure 23 shows the velocities of plate waves normalized to the velocity of bulk transverse waves in the medium.

3. Acoustic Device Materials and Fabrication

Most acoustic sensors employ a piezoelectric material to convert energy from electrical to acoustical form and vice versa. The name piezoelectric, referring to pressure-produced electrical charge, describes solids that develop a polarization charge when stressed or strained. The most important piezoelectrics are naturally occurring crystalline quartz (SiO_2), the artificial ferroelectric crystal lithium niobate (LiNbO_3), the sintered ceramics (such as lead-zirconium-titanate, usually abbreviated as PZT) that contain metals and the titanate ion, deposited films of zinc oxide (ZnO), and the polymer poly(vinylidene fluoride), also identified as PVDF.

The choice of proper piezoelectric for a given application involves many considerations. The ideal piezoelectric would have strong piezoelectric coupling—producing a large amount of polarization charge when a standard stress is applied—and it would have an acoustic wave speed that is unaffected by temperature changes. It would also be chemically inert, have low internal losses, and be both inexpensive and easy to form into devices. Table 7 lists some characteristics of the piezoelectrics actually in use.

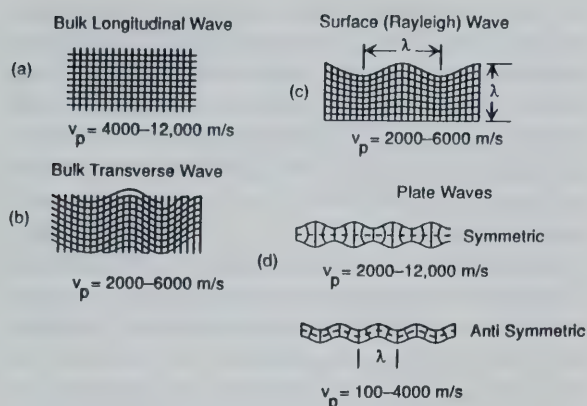


Figure 22. Pictorial representations of elastic waves in solids. Motions of groups of atoms are depicted in these cross-sectional views of plane elastic waves propagating to the right. Vertical and horizontal displacements are exaggerated for clarity. Typical wave speeds, v_p , are shown to right of each sketch. (a) Bulk longitudinal (compressional) wave in unbounded solid. (b) Bulk transverse (shear) wave in unbounded solid. (c) Surface acoustic wave (SAW) in semi-infinite solid, where wave motion extends below the surface to a depth of about one wavelength, λ . (d) Waves in thin solid plates (Lamb waves).

Some comments about entries in table 7 are in order. For decades crystalline quartz has been used to make the frequency-determining elements of stable electrical oscillators. The AT-cut and BT-cut quartz crystals vibrate in a shear mode and have a zero temperature coefficient of velocity at room temperature, 25 °C. When the crystal is cut at a

different precise angle with respect to its natural crystallographic axes to produce the so-called ST-cut, one obtains temperature-stable propagation of surface acoustic waves.

Commerically, thin films of zinc oxide—up to a few micrometers thick—have been formed by sputtering on glass to make high-volume commercial SAW filters used in television receivers. These films are deposited in an initially evacuated chamber that is back-filled with an ionizable gas, such as a mixture of argon and oxygen. Radio-frequency energy at the few-hundred-watt level supplied to the chamber causes ionization of the gas and bombardment of a source target of zinc oxide within the chamber. The thin film deposits at a rate of about a micrometer per hour on a substrate, such as glass or silicon blanks that are held in the chamber. Alternatively, zinc oxide may be formed by reactive sputtering from a pure metallic zinc source in a pure oxygen atmosphere. The zinc oxide deposits with its hexagonal crystalline axis normal to the substrate and so has a proper orientation for coupling piezoelectrically to bulk, surface, and plate waves. A degree of temperature compensation can be obtained with zinc oxide films by adding proper thicknesses of materials such as amorphous silicon dioxide.

Sputtering has also been used to produce thin titanate films, but here the substrate must be heated to about 600 °C instead of the modest 200°–250 °C required for zinc oxide. Recently, ferroelec-

Table 7. Typical piezoelectrics and their properties

Substance	Coupling	Temperature Stability	Other Considerations
Crystalline quartz	Low	High for special cuts: AT- and BT-cut for bulk shear waves, ST-cut for SAW	Not made in thin-film form; low loss; inexpensive; various modes can be launched
Lithium niobate	High	Low	Not thin film; expensive; low loss
Titanate ceramics	High	Low	Bulk or thin films possible (sputtering, sol-gel); require poling
Zinc oxide	Moderate	Moderate if compensated	Thin films (sputtering)
Poly(vinylidene fluoride)	Low	Low	Acoustic impedance match to water; requires poling; co-polymer can be cast.

tric and piezoelectric films of PZT have been formed by the sol-gel technique. In this process, the constituents are dissolved to make a fluid that is spun onto the substrate. Baking eliminates the solvent, after which electrodes can be fabricated lithographically and the film poled. To pole ferroelectric materials—the titanate ceramics and PVDF—one heats the material to above its ferroelectric Curie temperature, establishes a steady electric field in the substance by applying a potential difference across it, and then lets the material cool to below the Curie temperature. This aligns the ferroelectric domains and produces the required anisotropy.

Sheets of PVDF are commercially available for gluing onto a substrate. Recently a copolymer for use with PVDF has been described that can be spun or cast onto a substrate and so eliminate the gluing step.

Both SAW and plate-wave acoustic sensors can be made by using microfabrication techniques. The bad news is that microfabrication equipment can be quite costly: a “fab” line for making integrated circuits costs millions of dollars. The good news is that one can fabricate these acoustical devices with only a small amount of that equipment. The most expensive single piece of equipment for making sputtered zinc oxide or PZT films is the sputtering system itself, which might cost \$250,000 installed. The linewidths of most of the conductors used as the transducers shown in figure 21 are typically 5 μm or more, and these can be made with relatively inexpensive photolithographic systems. They could also be made in the type of commercial microfabrication “foundry” that is used by integrated-circuit manufacturers. To make the low-stress silicon nitride films presently used in flexural plate-wave devices requires a deposition system (either low-pressure or plasma-enhanced chemical-vapor-deposition, LPCVD or PECVD), in which nonstoichiometric nitride can be made (rather than Si_3N_4) by incorporation of excess silicon in the nitride as it forms.

4. Piezoelectric Transducers

The strength of the piezoelectric transducing effect in elastic-wave devices depends in part upon the configuration of the transducing electrodes used. Several types of transducer electrodes are shown in figure 21. To excite a bulk mode electrically, an alternating voltage is applied between broad-area electrodes on the two opposing faces of

a crystal plate (fig. 21a). Though it is hardly obvious, the piezoelectric stresses are generated at the interfaces of the plate with the conducting electrodes, which are usually one-half wavelength apart at the frequency of operation. Hence, the waves launched at each electrode add in-phase on traversing the thickness of the crystal, and resonant vibrations build up.

For SAW and plate-mode sensors, transducing electrodes are either interdigitated or unipolar comblike electrodes whose spatial period, p , equals the wavelength or some integral multiple of it (see figs. 21b and 21c). The strength of coupling of a given piezoelectric material in this situation depends on several factors:

- The piezoelectric and elastic properties of the material
- The orientation of the applied electric field with respect to the crystallographic axes of the material
- The ratio of spatial period, p , to operating wavelength, λ , and
- The relative locations of the comblike electrode and the ground plane, when a piezoelectric film is employed on a nonpiezoelectric substrate.

One can also use comb electrodes to launch bulk waves that travel into the interior of the solid to form the so-called acoustic plate-mode (APM) device of figure 21d. In this device, the orientation of the crystal is chosen so that the mode excited has particle motions that are entirely parallel to the broad surfaces of the device. This is known as the shear-horizontal, or SH, mode.

5. Phase Velocities and the Effects of Measurands

Most acoustic sensors detect a quantity to be measured—such as the number of vapor molecules adsorbed on the sensor—by its effect on the speed of the acoustic waves. There are several types of effect that can be used. We can understand two of these by noting that analyses show that acoustic-wave phase velocities, v_p , are typically related to the density and stiffness of the material as $v_p \approx \sqrt{\text{stiffness}/\text{density}}$.

Thus we can make gravimetric acoustic sensors, like the vapor sensor just mentioned, in which an addition of mass to the device causes a measurable

decrease in wave velocity. If something stiffens the device, the wave velocity increases; the polymerization of a liquid on the sensor could cause such an effect. It can be shown that the piezoelectric effect also stiffens a solid slightly, causing a velocity increase. For instance, in SAW sensors, if the piezoelectric fields associated with the traveling wave can interact with an adjacent medium, an increase in that medium's electrical conductivity produces a shielding effect, lowering the piezoelectric stiffening and hence lowering the wave velocity.

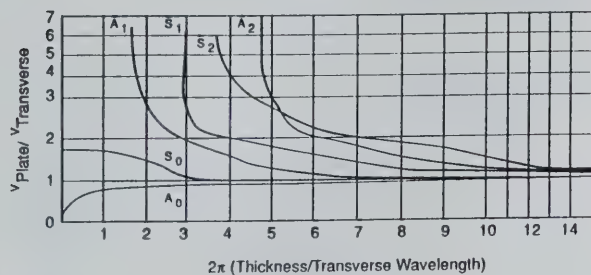


Figure 23. Plate wave velocities for symmetric (S) and antisymmetric (A) modes in an isotropic solid as a function of plate thickness. Poisson's ratio is assumed to be 0.34. (After fig. 32a in Reference 5.)

A very different effect can be used to sense viscosity with acoustic sensors. Here we can measure wave dissipation (loss) caused when the wave causes motion of an adjacent viscous medium. Analysis shows that loss varies with the square root of the viscosity.

Additional sensitivities can arise. For example, increasing temperature causes thermal expansion that increases the spacing between transducing electrodes in all the devices of figure 21, effectively increasing the wavelength of their operation.

Let us now consider how these changes of velocity or dissipation might be detected. Figure 24 summarizes the options. As the heart of the sensing instrument we may use either a resonator or a delay line. For example, figure 21a showed a bulk-wave resonator, and figure 21b showed a SAW delay line. We may incorporate either device in a passive circuit, where we measure the characteristics of the device itself, or we may connect it in an active circuit that provides electrical feedback to form an oscillator.

For passive bulk-wave resonators we measure resonant frequency, f_{res} , and infer the velocity from the relation $v = f_{res} \lambda_{res} = f_{res} d / 2$, where λ_{res} is the wavelength and d is the resonator thickness. For a passive delay line, changes of the phase shift be-

tween input and output electrical signals are used to determine changes of wave velocity.

For resonator or delay-line oscillators, the oscillator frequency is the output variable. It can be measured easily and precisely with a digital counter. For investigating wave dissipation, such as that caused when a viscous liquid contacts one of these sensors, we have several options. We can measure the quality factor of the resonator (or equivalently, the frequency bandwidth of the resonance), the insertion loss of the delay line, or the gain of the active circuit used for the oscillator.

Figures 21b and 21c show delay lines for SAWs and plate waves, but one can make resonators for those two types of waves rather simply in the following way. It is known that these waves are partially reflected upon encountering an obstacle or region where the wave velocity changes. By spacing a number of partial reflectors at equal center-to-center distances of one-half wavelength, one can produce a reflecting array that produces an almost complete reflection. By surrounding an interdigital SAW or flexural-wave transducer with two such reflecting arrays, one can form an acoustic resonator having a very well-defined and sharp resonance characteristic.

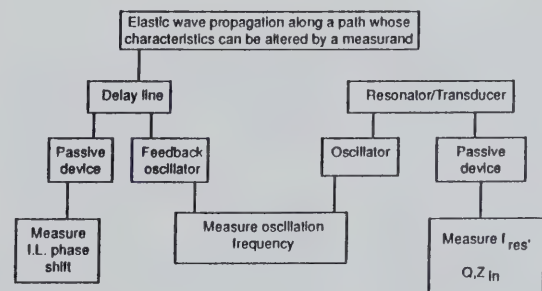


Figure 24. Measurement options for ultrasonic resonators and delay lines.

6. Gravimetric Response

We will now discuss the response of gravimetric acoustic sensors and compare devices using different types of waves.

In bulk-wave sensors, adsorption of gas molecules occurs on the surfaces of the crystal, which is typically connected to an oscillator circuit. Let us suppose that the mass per unit area, Dm , added as a result of vapor adsorption, is a small fraction of the original mass per unit area of the

crystal plate itself. Then the added loading causes the resonant frequency of the resonator to shift from its original value, f_{res} , to a new value, f_{loaded} . The change is an amount $\Delta f = f_{\text{loaded}} - f_{\text{res}}$, where

$$\Delta f / f_{\text{res}} = S_m \Delta m.$$

The factor of proportionality, S_m , is a negative quantity that depends upon the resonator material and the operating frequency or wavelength. For example, Lu and Lewis [6] found that the frequency of a 6-MHz, AT-cut quartz crystal resonator decreases 1 Hz upon sorption of 12 nanograms of gas molecules per square centimeter onto one of its faces.

For SAW sensors there is usually no analytic expression for phase velocity. Approximate analysis, based on slightly perturbing the SAW delay line by the addition of a layer to the surface, shows that a layer can both “mass load” the device and also stiffen it. Mass loading lowers the velocity, and stiffening increases it. Often the stiffening is so small that it can be neglected, allowing the frequency shift for a SAW delay-line oscillator to be written in the same form,

$$\Delta f / f_{\text{res}} = S_m \Delta m,$$

where S_m is determined from a perturbation analysis of the SAW.

One finds that when both a bulk-wave resonator and a SAW delay-line oscillator are operated at the same wavelength, the devices have comparable gravimetric sensitivities, S_m . The advantage of SAW sensors over bulk-wave gravimetric sensors lies in our ability to fabricate SAW sensors that operate at much shorter wavelengths—and hence much higher frequencies—than bulk-wave resonator sensors. This is because bulk-wave resonators designed for a very high resonant frequency become impractically fragile, because the thickness of the crystal is equal to one-half the wavelength.

Let us now examine the SAW delay-line oscillator more closely (see fig. 25). For coherent oscillations to establish themselves from circuit noise when the amplifier is turned on, at some frequency in the operating band of the transducers the total phase shift around the loop, consisting of delay line plus amplifier, must be an integral number of 2π radians. If the amplifier gain is sufficient to make up for coupling and propagation losses, the device will oscillate at the frequency f_{osc} , which is related to the wave velocity and other parameters by the equation

$$F(2\pi f_{\text{osc}} v)L + \Phi_E = 2\pi N,$$

where L = center-to-center distance between transducers, F_E = phase shift of amplifier (normally small), and N is an integer that is roughly equal to the number of wavelengths in the device (typically between 50 and 200). The important conclusion is that when the velocity of the SAW changes because of mass-loading or stiffening, the oscillation frequency must change a proportionate amount for the equation to remain valid. Thus, by monitoring frequency we monitor the wave velocity.

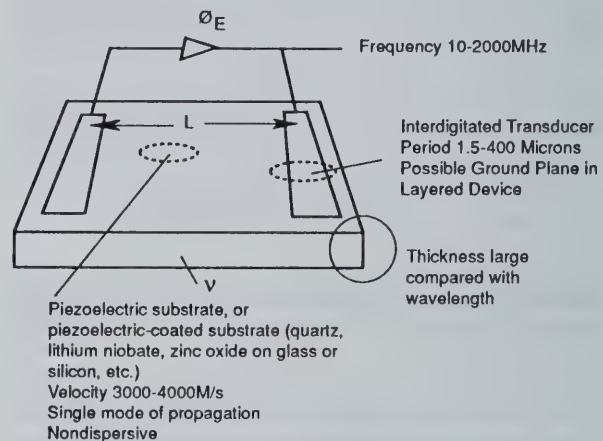


Figure 25. Surface acoustic wave (SAW) oscillator.

The plate-mode sensor can be understood by following similar reasoning. The plate-mode device of figure 26 has a thin nonpiezoelectric membrane (typically made of low-stress silicon nitride) with a thinner piezoelectric layer (typically sputtered zinc oxide). Comblike electrodes are formed photolithographically on the membrane, which has a ground plane between the piezoelectric layer and the supporting membrane.

With extremely thin membranes, very low A_0 -mode velocities are obtained. For example, with a $3 \mu\text{m}$ thick membrane and $100 \mu\text{m}$ wavelength, the flexural A_0 -mode velocity is about 400 m/s. This velocity is smaller than the speed of sound in most fluids (for example, the speed in water at room temperature is 1480 m/s). Thus, when the flexural-wave device is immersed in a nonviscous, ideal fluid, the wave produces only an evanescent disturbance in the fluid, and no energy radiates away from the membrane. (The behavior is similar to that of light in fiber-optic lightguides: the core of the fiber has a higher index of refraction than the surrounding cladding so the light travels more

slowly in the core than in the cladding, and the energy remains inside.) This is in sharp contrast with a fluid-immersed SAW sensor, whose acoustic energy radiates into the fluid because the SAW velocity is typically much higher than the velocity of sound in the fluid. The calculated rate of loss is about 1 dB per wavelength [5]. This loss is particularly serious for biosensors, which usually require contact with a liquid.

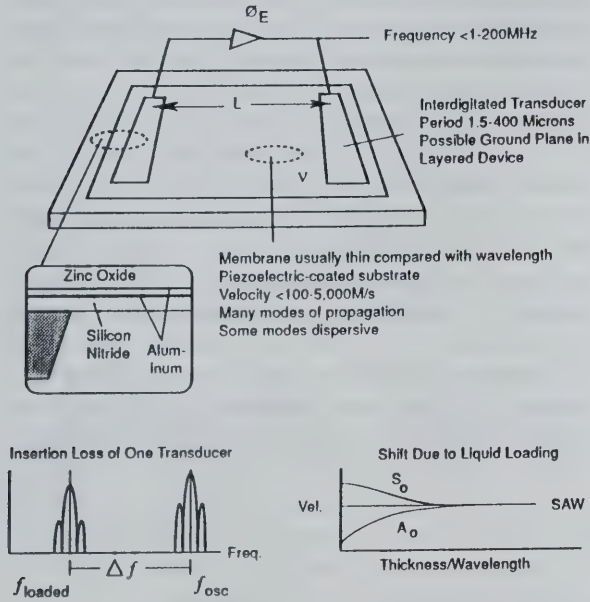


Figure 26. Flexural plate-wave (Lamb wave) oscillator.

One can show [7] that when the plate-wave velocity is much smaller than the fluid velocity, the evanescent decay distance, δ_E , is approximately equal to $\lambda/2\pi$. For a typical flexural-wave device having a 100 μm wavelength, this means the acous-

tic disturbance extends only about 16 μm into the liquid. One finds that when a large volume of fluid having density P_f contacts the membrane, its effect is equivalent to increasing the mass per unit area of the membrane itself, M , by an amount $\rho_f \delta$, the mass per unit area of a layer of fluid whose thickness is the evanescent decay distance.

The gravimetric sensitivity factor expressions for bulk, surface, and flexural plate-mode sensors are collected in table 8, together with experimentally determined values of S_m . The bulk- and surface-mode device sensitivities are inversely proportional to the wavelength, λ , while that of the flexural plate-wave device is proportional to the inverse of the plate thickness. It is possible to make a membrane having a submicron thickness but quite difficult to generate SAWs with wavelengths much smaller than a few microns. Hence, we can make plate-mode sensors that are more sensitive than SAW sensors. These flexural-wave devices will also operate at much lower frequencies, as well, because of their very low wave velocities. Computer simulation [8] supports this conclusion.

Unpublished estimates of the biosensing capability of the flexural-wave device show that one can expect a minimum detectable mass loading in water of about 500 picograms per square centimeter. Such a figure is obtained by choosing dimensions that optimize sensitivity and using the observed short-term oscillator noise observed with these devices, which is around 1 part in 10 million. Since these devices may be as small as 1 mm on a side, the estimated minimum detectable added mass is about 0.5 picogram.

Experiments have demonstrated the ability of the flexural-wave device to detect nonselective adsorption of protein molecules and bacteria contained in 10-microliter volumes of buffered saline

Table 8. Mass sensitivities S_m of bulk-, surface- and flexural plate-mode sensors. Here ρ , σ , and d are the density, Poisson's ratio and thickness of the device, respectively, and λ is the wavelength. The factor $K(\sigma)$ ranges from 0.8 (for $\sigma=0.5$) to 2.2 (for $\sigma=0$). (See footnotes for table 1 from Wenzel and White [9] for original sources of data.)

Sensor	S_m (theory)	Device	Freq. (MHz)	S_m (calc) (cm^2/g)	S_m (meas.) (cm^2/g)
Bulk wave	$-2/\rho\lambda$	AT-cut quartz resonator	6	-14	-14
Surface wave	$-K(\sigma)/\rho\lambda$	ST-cut quartz SAW delay line	112	-151	-91
Flexural plate	$-1/2\rho d$	ZnO-on-silicon nitride delay line	2.6	-951	-1014

solution placed on the uncoated surface of the sensor. Continuing research at the Berkeley Sensor & Actuator Center is aimed at demonstrating selective gravimetric sensing with a similar device.

6.1 Obtaining Selective Gravimetric Response

The most frequently asked question regarding gravimetric acoustic sensors is, how do you make them selective? A number of different approaches have been proposed and taken to achieve selectivity.

Most sensors employ two devices: an active and a reference device. Both are exposed to the same environment. Ideally, these two respond similarly to all extraneous influences, such as ambient temperature and pressure, while the active device alone responds to the measurand of interest. With ultrasonic oscillator sensors, the unwanted responses are canceled by subtracting the frequency of the active device from that of the reference device. This is either done digitally with frequency counters or by combining the two output signals in an electronic mixer whose difference frequency becomes the output signal of the sensor.

The entries in table 9 are methods used to differentiate the active from the reference device. For example, the active device might have a porous coating that permits entry only of molecules smaller than a certain size; larger molecules would be adsorbed on both the active and the reference device, and so have their gravimetric effects canceled when the outputs are mixed. Martin et al. [10] have demonstrated that with a porous sorptive coating one can also distinguish molecular diameters by considering the relative rates of diffusion into the coating: small molecules diffuse faster, causing a more rapid decrease of oscillator frequency than do large molecules.

Another approach is to match the absorber to the target molecule to be sensed by choosing the sorbant layer to have a solubility parameter near that of the target substance. Selective response can also be based on use of a chemical reaction that is selective for one compound, or a class of compounds of which only one member is likely to be present [11].

In gravimetric biosensing, it may be possible to obtain a high degree of selectivity. An example is the binding of an antibody to the corresponding

Table 9. Approaches for obtaining selective gravimetric response

Approach	Example
Active and reference device	Coated and uncoated SAW devices used together null out influence of ambient temperature
Filtering	Layer of zeolites permits volume sorption of molecules smaller than a certain size
Rate of diffusion	Organic molecules cover pore walls in porous zinc-oxide-coated SAW resonator at rate inversely related to molecular size
Solubility parameter	Xylene and acetone dissolve in a photoresist layer on SAW sensor while water does not
Chemical reaction	In SAW sensor, styrene vapor absorbed in polymer containing a metal-substituted compound selectively displaces ethylene, causing net change of mass per unit area
Biochemical reaction	Antigen and corresponding antibody bind in a bulk-wave or SAW sensor
Spectroscopy	Thermal desorption occurs in SAW sensor whose temperature is varied by resistive heating while output frequency is observed
Pattern recognition	Multiple SAW sensors having somewhat selective coatings respond differently to various vapors, permitting computer analysis of vapor present

antigen, which is nearly, but not entirely, selective. As noted earlier, the flexural plate-wave device is inherently well suited for operation in a liquid, because of its low wave velocity. Because that device is also quite sensitive gravimetrically, we expect that it will find use as a selective biosensor. To form such a device, one would bind selective receptors to the plate, a process used in other types of biosensors with reasonable success and yield. As corresponding biomolecules, present in a fluid specimen placed on the sensor, join onto immobilized receptor molecules, a frequency shift occurs. A recent article [12] that focuses on gravimetric sensing with bulk-wave crystals discusses aspects of this type of biosensing and gives some experimental data on responses observed.

Spectroscopy—observing sensor response while varying some parameter such as device temperature—permits us to obtain from a single sensor much more than a single item of information. Distinctive behavior may help us to identify the substance causing the response.

Even if a truly selective response cannot be achieved, an effective approach may be to apply pattern-recognition analysis to the response of a group of sensors having different nonselective responses [13].

7. Performance of Acoustic Chemical Vapor Sensors

To date, the most extensive investigations of chemical vapor detection with acoustic sensors have been made on SAW delay lines and resonators [14]. These have included devices made of ST-quartz and SAW delay lines and resonators made on silicon, which is not piezoelectrically active. (Zinc oxide films have been used for coupling.) Detection of gas concentrations as low as 10 parts per billion have been reported, in the case of H₂S [15]. Recent vapor-sensing measurements with a 3MHz flexural-wave sensor yielded a minimum detectable toluene concentration of about 70 parts per billion [16]. A number of the results are listed in table 10 and in a recent review article on SAW vapor sensors [17].

SAW gas sensor results are available [26] for the following vapors and gases: hydrogen, oxygen, halogens and halogenated hydrocarbons, hydrogen sulfide, sulfur dioxide, ammonia, nitrogen dioxide, organophosphorus compounds, styrene, cyclopentadiene, and water. Generally these devices are not selective, but selectivity has been observed in sensors involving a chemical reaction. A SAW styrene-vapor sensor [27] employed a sorptive film

Table 10. Responses of various SAW sensors

Type	Detection Characteristics	Reference
Chemical Vapor		
Any vapor	Limit = 3×10^{-11} g/cm ³ at 3 GHz	Wohltjen [18]
Dimethyl acetamide	358 Hz/ppm at 294 MHz; selective	Wohltjen [19]
H ₂ S	To 10 ppb using activated WO ₂	Vetelino et al. [20]
NO ₂	To 500 ppb, 100 Hz/ppm at 40 MHz with phthalocyanine; selective; mass loading and conductivity change	Venema et al. [21]
Mechanical		
Acceleration	1400–10,000 Hz/g at 105 MHz; response to 10^{-5} g	Hauden [22] Motamedi [23]
Pressure	400 Hz/kPa at 105 MHz, temperature-compensated –40 °C to 80 °C	Hauden [24]
Thermal		
Temperature	2–3 kHz/K, 10^{-4} °C resolvable	Hauden [25]

containing a compound that reacted with styrene and releasing ethylene. The coating on this device could be regenerated by flowing ethylene past it. Selective response has been observed for NO₂ with metallophthalocyanines in which a selective chemical reaction can occur. The metallophthalocyanines consist of central metal ions surrounded by a number of organic ions or molecules. The coating may be applied by the Langmuir-Blodgett dipping technique [28].

We will now summarize briefly some issues relevant to the performance of SAW sensors.

7.1 Minimum Detectable Concentration

Several factors come into play when one determines the minimum concentration that a vapor sensor can detect. These are: the sensitivity, as given here in terms of the factor S_m ; the noise level of the sensor that is typically set by random fluctuations of the wave velocity and by electric circuit noise; and the partitioning of vapor between the atmosphere surrounding the sensor and the sorptive film on the sensor. Conventionally, the detection limit is taken to be the concentration that produces a signal-to-noise ratio of 3. The partition coefficient that characterizes the third factor just mentioned depends upon both the vapor and the sorptive material used.

7.2 Coating Considerations

For fast response, one seeks a coating into which the vapor diffuses rapidly. For polymeric coatings, this means using a sorptive coating material at a temperature below its glass-transition temperature, T_G . For many polymers, values of T_G are found in handbooks. The thicknesses of the coatings applied to acoustic sensors are often specified in terms of the mass-loading shift of device frequency that the coatings produce when they are applied. (Thus one may speak of a "10-kHz coating.") Coatings can be applied in many ways, such as spraying, dipping, and the elegant technique of Langmuir-Blodgett film preparation that produces monomolecular films, solvent casting, and even localized deposition by means of a modified ink-jet printer [29].

7.3 Temperature Stability

ST-cut quartz SAW delay lines that operate at a few hundred megahertz may have velocities that vary randomly by only 1 part in 10 million or 100 million. SAW resonators made of ST-cut quartz

can be even stabler. When coated, the devices usually are less stable (noisier), but practical coated SAW devices may nevertheless exhibit instabilities around 1 part in 10 million.

8. Physical Measurements with Acoustic Sensors

The preceding section dealt exclusively with chemical vapor and gas sensing because that topic is relatively well contained and of interest to a particular audience. The acoustic sensors can also be used to measure a wide variety of physical quantities, including differential and absolute pressure, acceleration, fluid density, and viscosity. We will now summarize those capabilities.

As indicated above, these devices will respond to anything that changes either the speed or the attenuation of the acoustic waves. This has been used in some commercial and research or development devices that respond to purely physical measurands such as pressure and acceleration. (Some of the results obtained with SAW sensors were listed in table 10.) The advantages of using acoustical devices for these purposes are twofold: (1) Sensors such as SAW oscillators are inherently very sensitive, and their dynamic range can be quite large, in part because the output variable is a frequency whose small changes can be measured with great precision. (2) For applications requiring stability in spite of changes of ambient temperature, one can use thermally stable SAW resonators made of ST-quartz.

The flexural plate-wave sensor responds quite sensitively to mass-loading of its surface, because the mass of its plate itself is small. This sensitivity is illustrated by the observed reaction of the device to contact on one side with water: the frequency of a 3 μm thick device operating in air at about 5 MHz dropped by a full 36% (see table 11). Not only can this effect be used to measure the density of liquids, but it can also be used to make measurements of absolute gas pressure. When the same device was placed in a pressure chamber containing helium, a frequency change of about 1.5-kHz-per-atmosphere pressure change was observed. In this situation, the sensor experiences no net force on its thin membrane, while it responds to pressure-induced changes of gas density. In another test of the device where a pressure was applied to only one side of the thin plate, a differential pressure response of about 0.7 Hz per microbar was found.

We described earlier how one could detect gravimetrically the addition of mass to an acoustic sensor, for example by sorption of vapor molecules or plating onto the surface of the device. One can also detect removal of a mass with such a device, and so follow the processes of etching or corrosion of a surface film.

A most interesting application of these devices is to measure the viscosity of fluids. For satisfactory operation of these acoustic devices in fluids, either of two conditions should be met. One possibility is to use an acoustic mode whose particle motion is parallel to the fluid-device boundary, so as to avoid setting up compressional waves in the fluid that would carry the wave energy entirely away from the sensor. This option means using shear bulk-wave devices or the acoustic horizontal-shear plate-mode (APM) device of figure 21d. The other option, mentioned earlier, is to use the flexural A_0 mode

whose velocity is lower than the velocity of sound in the fluid. Then only a nonradiative evanescent disturbance is set up in the fluid.

Analysis shows [30] that the loss suffered by the waves in either APM or flexural-wave delay lines is proportional to the square root of the viscosity of the fluid. This dependence has been verified experimentally in tests with a series of water-glycerol solutions of varying concentration. In acoustic viscometry, it is advantageous to use a low wave frequency in order to extend the range of the device to high viscosities. This is because viscous substances behave as if they were elastic when acted upon by waves whose period is shorter than the viscoelastic relaxation time for the substance. It appears possible to measure viscosities as high as 10,000 or even 100,000 centipoise with flexural-wave sensors that have relatively thick membranes. With thin-membrane devices, attenuation can be

Table 11. Sensor characteristics for ZnO-on-silicon flexural-wave devices. Overall device thicknesses ranged from 2 to 3 micrometers

Measurand	Freq. (MHz)	Characteristics	Reference
Biological			
Bovine serum albumin in buffered saline solution	5.8	Nonspecific adsorption on bare membrane; XHz/(mg/cm ²) calibration from electroplating copper onto membrane	Costello et al. [28]
Chemical			
Toluene vapor	2.85	12.2 Hz/ppm with ethyl cellulose coating 0.5 μ m thick; 70 ppb estimated minimum detectable concentration	Wenzel and White [29]
Mechanical			
Pressure—differential	4.7	0.65 Hz/microbar	Wenzel and White [30,31]
Pressure—absolute	4.7	1.5 kHz/atm in helium	Wenzel and White [31]
Density of liquid	4.7	One-sided loading with de-ionized water causes 36% drop in frequency	Wenzel and White [31]
Viscosity of liquid	4.7	Strongly dependent on plate thickness; 56 dB added loss for glycerol-water solution having viscosity of 1060 centipoise	Costello et al. [28]
Thermal			
Temperature	4.7	– 120 ppm/°C for bare device	[30,31]

correlated with viscosity for relatively small viscosities. Viscosities of some familiar substances are: water at 20 °C, 1 centipoise; human blood, 5 to 12 centipoise; pure glycerol at 20 °C, 1412; lubricating oil SAE No. 10W at -18 °C (0 °F), 1200–2400 centipoise.

9. Kinetic Effects of Flexural Waves

We should note finally that interesting and useful kinetic effects have been produced with the flexural plate-wave devices that also function as sensors. These effects—which might be termed “microtransport”—include moving small-fluid volumes that contact the plate carrying the ultrasonic waves, or causing small granular solids such as polycrystalline silicon blocks (and even particles of nondairy creamer!) to move over the surface of the plate. In addition, appreciable mixing of fluid near the plate has been detected.

While these new effects are still being explored and analyzed, several features are clear. The velocity of motion increases as the square of the wave amplitude, suggesting a nonlinear origin for the effects. The likely candidate is a nonlinear phenomenon known as acoustic streaming [31]. Pumping of water with 5-MHz ultrasonic plate waves has been observed at velocities up to 100 micrometers per second. While that may seem like a small velocity, substantial and useful flow volumes could be obtained if a large number of such ultrasonic micropumps were used in parallel.

The RF drive voltages used are conveniently low—typically no larger than 10 volts. Furthermore, an electrically insulating membrane separates the drive voltages from the fluid pumped—an important consideration in certain biosensor applications or when one must deal with electrically conducting fluids. The thinness of the membranes in which the acoustic waves travel is responsible in part for the dramatic effects observed: the few milliwatts of acoustic power coupled into the device are traveling in a very thin plate and so large particle motions result. Surface displacements as large as 100 nm have been measured. These are large enough so that the stationary patterns of standing plate waves can be observed visually in an optical microscope outfitted with a Nomarski interference attachment. The ability to “see” the waves should facilitate design of both flexural-wave sensors and kinetic flexural-wave devices.

10. Business Opportunities Involving Acoustic Sensors

The foregoing suggests a number of attractive characteristics of the types of acoustic sensors discussed here:

- They are versatile, sensitive, and relatively simple to make
- Their output—a frequency—is readily handled by digital electronic circuitry
- Some of them—particularly flexural-wave devices—operate at conveniently low frequencies with low-cost auxiliary equipment.

There are also manufacturers who can make the acoustical devices that are the heart of the sensor systems (see partial list below in sec. 11).

At the start of the 1990s, there are only a few companies making acoustic sensors. What is most needed for an increase in this number is identification of large-volume uses for these devices. For a dedicated microfabrication facility to be economic, millions of units per year must be made and sold, and to date, no such large market has been found for acoustical sensors. It should be noted, however, that the vapor-sensing capabilities of these devices have been studied for many years because of their relevance to detecting agents that might be used in chemical and biological warfare.

Applications one may envision include chemical vapor sensing (detecting airborne pollutants and toxic releases in the work place or atmosphere), monitoring the densities of fluids (in chemical process control, medical monitoring), measuring viscosity of very small fluid volumes (monitoring lubricant quality, medical uses), and detecting contaminating chemical components in fluid streams (process control, oceanic monitoring). When selective biochemical detection is achieved, a possible application for these devices is in real-time testing in physicians’ offices for biomolecules such as antibodies or DNA fragments. The ability of the flexural-wave devices to sense and move fluids and granular solids could result in their being used in systems that pump, mix, and ultimately also meter the delivery of small quantities of fluids. An example would be a system that reconstituted dried drugs for delivery into an intravenous tubing in a hospital.

An entirely different set of markets that may be attractive is the instrumentation segment. An example is the commercialized bulk-wave crystal-deposition monitor that is commonly found in vacuum evaporators used for deposition of thin aluminum films during IC fabrication. The instrument consists of a replaceable quartz crystal resonator in a water-cooled holder inside the evaporator chamber and a microprocessor-based controller. Aluminum evaporated from a heated source deposits on both the silicon wafers in the evaporator and the quartz crystal, lowering the oscillator frequency proportionately. Deposition thickness and deposition rate are indicated on the instrument's display; a computer interface is also available for control or data logging.

One can imagine other successful instruments that are similar to the deposition monitor. With the flexural plate-wave sensor one could make a fluid densitometer having part-per-million resolution. Either that or the acoustic plate mode device can be used as a viscometer; both require only microliter volumes of fluid to test, in contrast with conventional viscometers. Any of the four basic acoustic devices of figure 21 could be used to monitor corrosion of films deposited on them. Observation of the polymerization of polymeric liquids as they "set" and determination of the glass transition temperature of polymers are other applications. Weighing very small objects may be another application for these devices.

The problem of nonselective response may be solved by making arrays, as mentioned earlier, or by obtaining selectivity by elution, as is done in gas chromatography, and using the acoustic sensor at the output as a nonselective but very sensitive detector of the arrival of a sample volume.

11. Further Sources of Information

The references cited in this chapter identify many of the researchers in this field, and the publications themselves identify the organizations, whether industrial, educational, or governmental. New research in the field is reported in many journals, including *Analytical Chemistry*, *Sensors & Actuators*, *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control*, and *Applied Physics Letters*. A collection of articles on the subject appeared in a Special Issue on Acoustic Sensors, *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control*, vol. UFFC-34, No. 2, March 1987. Conference presentations on

the subject are made at the IEEE Ultrasonics Symposium each fall, the Hilton Head Workshop on sensors and actuators in June, the biennial Transducers '87, '89, '91, ... series, and at other topical conferences.

On the topic of microsensors generally, there is available from the IEEE Service Center, 445 Hoes Lane, Piscataway, NJ 08854, an IEEE Reprint Volume entitled "Microsensors." Also you can obtain, for either Apple Macintosh or IBM-compatible personal computers, an inexpensive diskette containing over 2000 citations to microsensor references from the published literature and from conferences. For information about the diskette, contact Dept. B, University of California, Extension 2223 Fulton St., Berkeley, CA 94720.

Among the companies that manufacture acoustic devices that can be used as sensors are the following:

Sawtek, Inc., P. O. Box 18000, Orlando FL 32860.
RF Monolithics, Inc., 4441 Sigma Rd., Dallas, TX 75234.

Complete SAW sensors and sensing systems are made by:

Microsensor Systems, Inc., 6800 Versar Center, Springfield, VA 22151.
Integrated Chemical Sensors Corp., 44 Mechanic St., Newton, MA 02164.
Xensor Integration bv, Mekelweg 4, 2628 CD, Delft, The Netherlands.

Piezoelectric crystals are available from many companies including:

Crystal Technology, Inc., 1035 E. Meadow Circle, Palo Alto, CA 94303.
Valpey-Fisher Corp., 75 Smith St., Hopkinton, MA 01748.

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with particular emphasis in ultrasonics and sensors. White's publications and patented inventions concern sensors, ultrasonic phenomena and devices, thermoelectric effects and microwave electronics. Among his awards are the IEEE Cleo Brunetti Award (1986), a Guggenheim Fellowship (1968), and the IEEE UFFC Society's Achievement Award for contributions to the field of ultrasonics in photoacoustics, surface acoustic wave devices, and sensors. Professor White is Co-Director of the Berkeley Sensor and Actuator Center, an NSF/Industry/University Cooperative Research Center. He is also the 1989-90 Distinguished Lecturer of the IEEE Ultrasonics, Ferroelectrics and Frequency Control Society.

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Oxygen, Glucose, Urea, and Cholesterol Sensors for Biomedical Application

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There is a constant need for simple, quick, and inexpensive physiological measurements close to the patient. Although transducers and sensors have been widely used for the measurement of common physical phenomena—for example, flow, pressure, and temperature—any such developments in the measurement of chemical species has been very slow. Sensors have been developed for several species of biological importance such as oxygen, carbon dioxide, pH, potassium, glucose, urea, creatinine, and cholesterol but only a limited few have been commercialized. This chapter describes sensors for four of these biological species: oxygen, glucose, urea, and cholesterol. The first three

sensors could be used both for continuous measurements for which no comparable techniques exist, and for discrete measurements as an inexpensive alternative to existing technology. A cholesterol sensor is needed only for discrete analysis. I discuss here the operating principle of these four sensors, their development status, and the problems that need to be solved before they can become commercial successes. Because the electrochemical approach is the farthest developed, only sensors based on this technique will be described.

Key words: amperometric; biosensors; cholesterol; enzymes; glucose; in vitro; in vivo; membranes; oxygen; urea.

1. Introduction

Frequent measurement of biochemical parameters—such as blood cations, gases, sugars, hormones, and other metabolites—is essential to the delivery of optimum patient care. Several automated systems have now been developed that improve the accuracy and precision of these measurements and at the same time reduce the cost per analysis. However, they have not changed the underlying principles of analytical techniques. Bringing these systems closer to the patient would offer the potential for better patient-care information with significant cost savings. In addition, the labor-intensive specimen collection, processing, and reporting steps (which in many cases cost more than the analytical procedure) could be avoided. This need for bringing the analytical systems close to the patient could be better achieved by combining the principles of biochemistry and mi-

croelectronics, resulting in what is called a “biosensor.”

Biosensors are essential in medicine for two different applications. First, they are necessary for continuous in vivo monitoring of certain species whose concentration changes so rapidly that in vitro analysis would be inadequate to follow its time course using conventional assays. Examples in this category are oxygen, carbon dioxide, pH, glucose, and potassium. Second, they are useful for rapid, simple, and inexpensive in vitro analysis of samples by relatively unskilled personnel at the patient bedside, in the physician's office, or at home to provide instant diagnostic data.

Deciding areas in the second category where biosensors will improve or replace an existing method is not an easy task from the commercial point of view, because user surveys yield conflicting

results in the case of several parameters. However, there is no doubt about the usefulness of sensors to measure oxygen, glucose, urea, and—probably to a lesser extent—cholesterol. The dire consequences of unrecognized hypoxia, and in the neonate of hyperoxia, has highlighted the need for close monitoring of oxygen [1]. The measurement of arterial oxygen concentration is helpful when compared with that of inspired oxygen. Glucose measurement is important for diabetes in order to adjust diet, activity, and insulin dosage for optimum therapy [2]. Urea is measured routinely to determine renal perfusion/function [3]. Although discrete analysis may be sufficient for this purpose, continuous urea measurement will be useful in dialysis patients to control the efficiency of dialysis. Accurate measurement of cholesterol is important, because it has been identified as one of the factors that contribute to heart attacks [3].

In this chapter, I will try to explain the operating principle of these sensors, the development status, and the problems that have, so far, impeded their commercialization.

2. Oxygen Sensors

2.1 Clinical Need

The determination of partial pressure of oxygen (pO_2) in blood is very useful as a diagnostic procedure in hospitals. In the U.S.A. alone, at least 25 million arterial blood samples a year are taken from patients to determine the partial pressure of oxygen.

Decreased arterial pO_2 can be caused by the following [3]:

- Decreased diffusion capacity of the alveolar lining membranes for oxygen (e.g., respiratory-distress syndrome in newborn infants).
- Decreased alveolar ventilation, either of peripheral origin (suffocation, submersion, paralysis) or of central origin (depression of the respiratory center by barbiturates or morphine).
- Increased venoarterial shunting of the blood (congenital heart failure with cyanosis). If the arterial pO_2 falls to a low level for only a short time, brain damage or death may follow.

Increased pO_2 in the arterial blood is caused by breathing oxygen-enriched air. The toxic effects of oxygen are especially pronounced in the newborn,

where oxygen treatment may cause fibrosis in the eye, with blindness. Therefore, it is essential to control the pO_2 in the newborn during oxygen therapy to avoid high concentrations for extended periods.

2.2 Commercial Availability

2.2.1 Sensors for Discrete Analysis The Clark oxygen sensor [4] became available to clinicians approximately 30 years ago and has become part of the most frequent in vitro diagnostic procedure in hospitals. This sensor works on an amperometric principle where the current resulting from the reduction of oxygen after applying a suitable potential is measured. Galvanostatic sensors based on the spontaneous reduction of oxygen by proper choice of the electrode pair without applying an external voltage has also been described. The galvanic probes have long-term stability of sensitivity but a finite lifetime compared to that of the amperometric method because of the gradual oxidation of the anode surface.

2.2.2 Sensors for Continuous Measurements The available sensors, based on either amperometric or galvanic principles, are capable of analyzing individual blood samples and thus give information at one point of time. They provide, that is, a snapshot of what is happening. Large variations in oxygen levels can occur within a few minutes and can lead to serious problems if not corrected immediately; hence, continuous measurement of oxygen is very useful. However, such sensors are not currently available. Both invasive and noninvasive sensors have been attempted for continuous in vivo measurements.

2.2.3 Noninvasive Measurements It would be ideal if some type of noninvasive technique could be developed for monitoring O_2 levels in blood. At normal temperatures, pO_2 at the surface of human skin is near zero. However, if the skin temperature is raised, the pO_2 at the surface can approach the arterial value [5]. Electrochemical sensors are now commercially available that measure the O_2 diffusing through intact skin. This method is called transcutaneous technique.

Transcutaneous gas monitoring has proved to be useful in the management of premature babies. Because it depends upon the skin being well perfused, it is unsuitable for use in patients in circulatory failure or shock [6].

2.2.4 Invasive Measurements Among the invasive sensors, only one sensor has gained clinical acceptance and is used in newborn intensive-care

units, particularly in the U.K. The device is built into the tip of a catheter and is used to monitor pO_2 in newborn infants. It is introduced into the aorta via the umbilical artery [7]. This method of monitoring O_2 through the umbilical artery is available only during the first few days of life while the umbilical artery remains patent. Hence, the need for developing an in vivo oxygen sensor that can be used in all cases still exists.

2.3 Characteristics of an Ideal In Vivo Oxygen Sensor

Site:	Ideally noninvasive, for example, transcutaneous
Size/shape:	If invasive < 20 G needle
Materials:	Biocompatible
Range:	0–760 mm Hg oxygen partial pressure
Stability:	72 hours
Operational stability:	2 mm Hg over 8 hours
Specificity:	No interference from anesthetic gases
95% response time:	30 sec
Calibration:	Every 8 hours
Sterilization:	Sterilizable
Operation:	Simple
Cost:	Inexpensive
Sensor shelf time:	At least 6 months

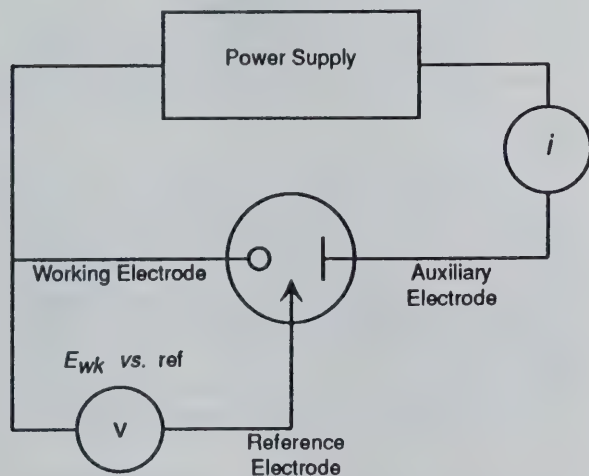


Figure 27. Schematic diagram of a three-electrode cell.

2.4 Principle of Operation of Oxygen Sensors

In the present chapter, I will discuss only amperometric oxygen sensors, even though they could be based on galvanic principles as well. The usual

three-electrode setup for the amperometric detection of oxygen is composed of a working electrode (anode), an auxiliary electrode (cathode), and a reference electrode connected to an amperometric detector (fig. 27).

When an adequate negative voltage is applied between the working electrode and auxiliary electrodes, oxygen is reduced at the surface of the working electrode, generating a current that can be measured. The potential at which the working electrode is held is measured with respect to the reference electrode. In many instances, the auxiliary and reference electrodes are combined into one, resulting in a two-electrode system.

Most commonly, the working electrode is a metal, such as platinum, gold, or silver. When this working electrode is biased at a negative potential with respect to a suitable reference electrode in a neutral potassium chloride solution, a current-voltage diagram called a polarogram is obtained (fig. 28).

As shown in figure 28, the current increases initially with an increase in the negative bias voltage due to the reduction of dissolved oxygen in the solution according to the equation:



The current that is passing destroys oxygen, changing its concentration close to the electrode surface. For the current to continue, oxygen has to be supplied to the electrode from the bulk solution. This happens mainly by two processes:

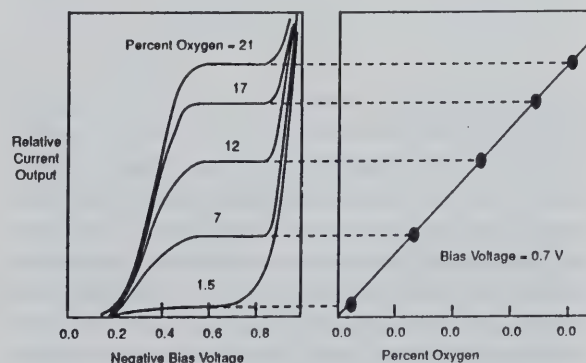


Figure 28. A typical polarogram and calibration curve for oxygen reduction.

- Convection. Convection results from the movement of the fluid either by forced means or from density or temperature gradient within the fluid. Convection moves the fluid from one point to an-

other, carrying along with it any dissolved species, including oxygen. This is the main mechanism of oxygen transport in the bulk of the solution except in a very thin layer close to the electrode surface. Experiments show that a thin layer of fluid, on the order of a few monolayers immediately close to a solid surface, remains motionless regardless of stirring the solution. For the electrode process, the reactants and products will have to move across this stagnant layer by diffusion.

- **Diffusion.** Oxygen is destroyed during electrochemical reduction, changing its concentration at the electrode surface (the rate of depletion depends upon the voltage). When the bias voltage is sufficiently negative, the reduction of oxygen is so fast that its concentration at the electrode surface is zero, and the current is determined wholly by the transport of oxygen from the bulk of the solution to the electrode, according to Fick's Law.

$J = D \, dc/dx$, where J is the flux of oxygen per unit area normal to the electrode surface and dc/dx is the concentration gradient of oxygen in the direction of diffusion.

Because the concentration of oxygen is primarily maintained constant in the bulk of the solution where convective flow is operational, the main concentration gradient occurs within the stagnant layer.

But in an unstirred solution, the thickness of the diffusion layer is not well defined, and it can extend out into bulk solution.

2.5 Membrane-Covered Polarographic O₂ Sensors

In theory, measurement of O₂ concentration should be possible using a noble metal electrode in conjunction with a reference electrode. However, in practice, it is necessary to cover the electrodes with a gas-permeable membrane for three reasons. First, electroactive species other than O₂ can react with or poison the electrodes. Second, if measurements are being made in situ, then changes in the electrolyte composition will change the electrode response, and frequent calibration will be necessary. The membrane separates the electrodes and the electrolyte solution from the test solution; hence, it gives a reproducible electrolyte solution. Third, the membrane provides a diffusion layer of controlled thickness at the electrode.

2.6 Design of Membrane-Covered Polarographic Electrodes

The schematic of a membrane-covered oxygen sensor is shown in figure 29. As mentioned above, the membrane-covered O₂ sensor consists of two electrodes: an electrolyte and a membrane that separates this electrolyte from the sample solution.

In designing O₂ probes, attention should be given to each component of the sensor: the electrodes, the electrolyte, and the membrane. All the requirements for the sensor performance should be clearly defined at the outset of the sensor design. For biomedical applications, the following parameters are kept in mind during design (refer to sec. 2.3 for the specifications):

- Sensitivity
- Specificity
- Operational stability and drift
- Response time
- Flow independence
- Sterilizability.

Sensitivity is mainly determined by the membrane material and its thickness. The speed of response is decided by the electrolyte reservoir, the membrane thickness, and the geometry of the detector. Specificity is a factor of the electrode material and the membrane. Stability is determined by the electrode's mode of operation, the electrode material, and the solvent medium.

2.6.1 Electrode Material For polarographic O₂ sensors, Pt, Au, and Ag have been used as the cathode and Ag/AgCl or calomel as the reference electrode.

The ideal polarographic sensor is one where the diffusion current plateau stretches over a wide range—that is, the output current is independent of applied voltage over the range. This wide current plateau is necessary to eliminate any error in measuring O₂ when the reference voltage is shifted for any reason.

A. Advantages/Disadvantages of Au versus Pt

1. Gold has a wider plateau than Pt [8]. The plateau is limited at the cathodic end by evolution of H₂ due to reduction of H⁺ ions and at the anodic end by the low catalytic efficiency of oxygen reduction.

2. The reduction of oxygen at gold is less complicated and the surface aging less pronounced [9].

3. Gold is less susceptible to poisoning by noxious gases, especially H_2S [9]. Pt has a high adsorptive capacity for many gases, even those as unreactive as methane. A sudden change of the partial pressure of such a gas will give a transient current.

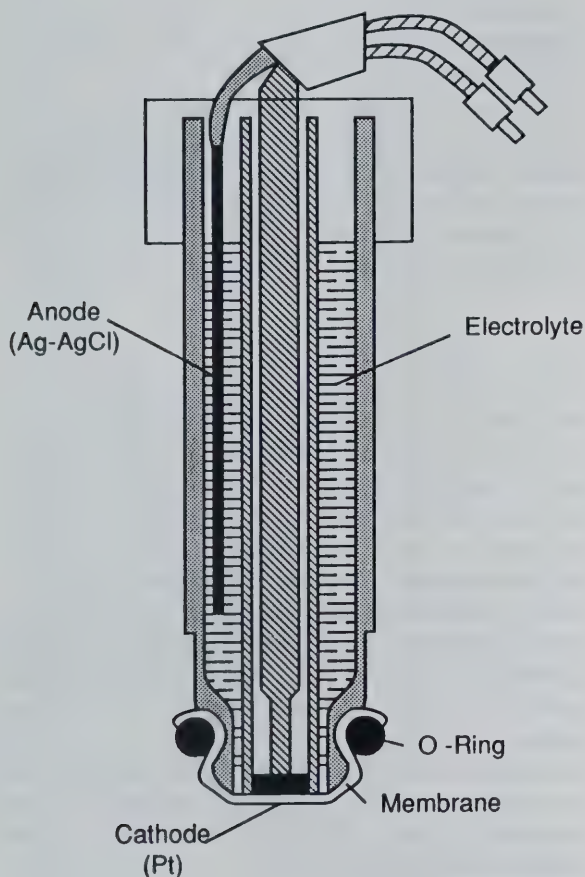
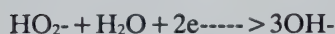


Figure 29. A membrane-covered oxygen sensor.

B. Advantages/Disadvantages of Au versus Ag

1. Au has a cathodic limit as high as that for silver; however, the waves show a shorter plateau because, unlike in silver, where O_2 reduction appears as a single plateau [10], in gold the reduction takes place in two steps:



2. Even if Au is used as cathode with Ag or AgCl as the reference, it is possible that some of the silver will be deposited on the cathode, especially in a two-electrode format. Thus, the catalytic activity of the cathode changes, resulting in change in current

output. In an Ag/Ag configuration, no such drastic change occurs.

3. One way silver is inferior to Au or Pt is in its lower resistance to corrosion, even though it will not be corroded during cathode operation. Electrolytes in which silver can form soluble complexes should be avoided.

4. Silver is a catalyst for anesthetic agent reduction; hence, oxygen sensors based on silver are subject to interference from agents such as N_2O and halothane [11].

2.7 Reference/Counter Electrode

In order to function as a reference electrode, an electrode must have a stable potential so that the reference does not change. Ag/AgCl is usually used as the reference electrode. Carritt and Kanwisher [12] used Ag/Ag₂O because it gave better stability to the electrode.

In many instances, the reference electrode also acts as the anode, resulting in current passing through the electrode. When the electrolyte contains Cl^- , the chloride concentration falls as it is being used by the anode reaction and OH^- is produced as a result of oxygen production and consequently change to Ag₂O. Because the reference voltages are different for Ag/AgCl (+0.222 V) and Ag/Ag₂O (+0.350 V), the voltage applied at the cathode with respect to the reference changes. Unless the current is independent of voltage, or at least covering the difference, gross error in the sensor output will result.

2.8 Electrolyte

The composition and volume of electrolyte are important parameters that determine characteristics of the oxygen sensor such as response time and stability of the response.

As far as the conditions for oxygen reduction at the cathode are concerned, the choice of the pH of the electrolyte is not so important, because the pH in the vicinity of the cathode soon becomes very alkaline due to OH^- formation, once the sensor is in operation.

Brookman [13] tested several electrolytes and concluded that only KCl and K_2HPO_4 gave a linear response to high oxygen tension. Because KCl gave a high residual current, he recommended using 1M K_2HPO_4 as the electrolyte. The electrolyte concentration is generally determined to give good conducting medium between anode and cathode.

If carbon dioxide is present in the sample, it can permeate through the membrane and change the pH of the electrolyte. Even though it should not be a serious problem for the reason mentioned above, Severinghaus [14] used 0.5 M NaHCO₃ + 0.1M KCl, which had pH of nine, to reduce this problem.

A rather serious problem associated with the electrolyte solution is the loss of solvent from the electrode. Depletion of the solvent leads to increases in the ohmic resistance of the electrolytic cell. This problem is especially serious since the electrolyte film is very thin because of the requirements for a fast response.

Several methods are used to reduce the solvent depletion. One method is to dissolve the electrolytes into a nonaqueous solvent with higher boiling points than that of water—for example, ethylene glycol. Ethylene glycol is hygroscopic and absorbs water from the surroundings. In addition, water can be retained by the addition of hygroscopic or deliquescent salts, such as LiCl or KH₂PO₄. Another less elegant method [15] has been to use a large electrolyte reservoir and transfer solvent from the reservoir to the electrolyte film.

2.9 Membrane

The membrane serves several purposes:

- It protects the electrodes from other electroactive or surface impurities.
- It obviates the need for frequent calibration for in situ measurements.
- It provides a finite diffusion layer of controlled thickness at the electrode.

Because the current output is directly related to the thickness and oxygen permeability of the membrane, the response characteristics are affected by the membrane properties.

An ideal membrane should have several characteristics:

- The membrane has to be mechanically strong and chemically inert.
- The membrane should have low oxygen permeability so that the membrane controls oxygen diffusion and high oxygen diffusivity for fast response.
- Water permeability should be low to avoid water loss.
- Ideally, the membrane should allow only O₂ to pass through it.
- The membrane should be biocompatible.

In principle, a variety of membranes—such as polyethylene [16], silicone [17], mylar [18], and teflon [19]—could be used. In practice, most of the workers have used either polyethylene or teflon for several reasons:

- Ready availability
- Mechanical strength
- High oxygen diffusion.

I have used [20] silicone rubber/polycarbonate copolymer from General Electric for our oxygen sensor because of its excellent biocompatibility. The thickness of the membranes commonly used at present is 10–25 μm.

2.10 Performance Characteristics

2.10.1 Selectivity Polarographic oxygen sensors achieve selectivity by a unique combination of the working electrode material, electrode potential, supporting electrolyte, the reference electrode, and the gas-permeable membrane. In any event, complete reaction specificity cannot always be attained, and side reactions may occur. Because a gas-permeable membrane separates the sample solution from the internal solution, no ionic species poses interference. However, other gases could diffuse through the membrane and pose an interference problem.

Any gases that have higher reduction potential than that for oxygen will also be reduced at the electrode. Oxygen sensors for in vivo application could have interference from anesthetic gases—especially from halothane [11].

Selectivity can also be achieved from membranes. The membrane acts as a filter simply because it will have different permeabilities to different gases [21]. The solubility of the interfering gases in the membrane should be minimum. Because the O₂ sensor operates in the diffusion limited condition, selectivity can also be achieved by nonsteady-state measurement.

2.10.2 Response Time The detector response time is mainly determined by the transport of the gas through the membrane:

$$t_{\text{sec}} = (\Delta X)^2 / D_m$$

t_{sec} = detector time constant in seconds

D_m = diffusion coefficient of the membrane for oxygen in cm²/sec.

ΔX = thickness of the membrane

A thinner membrane with high oxygen diffusivity is expected to give a rapid response time. Most of the oxygen probes show a marked difference between up-step response and down-step response [22]. These probes generally employed a large electrolyte close to the cathode. However, probes that had small amounts of electrolyte or whose electrolyte layer was located far from the cathode did not show such behavior.

2.10.3 Sensitivity The sensitivity limit of polarographic oxygen sensors is a function of the flux density of oxygen-reduction reaction in relation to any interfering side reaction or background reaction.

The detector reaction is related to the sensor parameters by the following equation:

$$i = (PA/\Delta X)$$

i = detector signal for a certain partial pressure of oxygen

A = diffusion cross sectional area

P = permeability of the membrane

ΔX = membrane thickness

The equation is a reasonable approximation of the detector signal, assuming that the mass transport through the membrane over the working electrode surface is one-dimensional and that the membrane limits the number of oxygen molecules that arrive at the electrode surface. Other factors being taken care of—a thinner membrane and/or a membrane with larger permeability coefficient—must give higher signal levels and sensitivity.

At zero oxygen concentration, a residual current is always present that could arise from a number of sources, such as reduction of some impurities in the solvent or electrolyte material. Also, oxygen dissolved in the insulating body of the sensor, especially if it is plastic, could diffuse out in an oxygen-free environment and contribute to a residual current. By minimizing the use of such plastics and purifying the solvent and electrolyte, the residual current can be minimized.

2.10.4 Linear Range From a medical standpoint, the oxygen concentration required to be measured varies over a wide range, from 0- to 760-mm Hg. Fortunately, a well-defined oxygen sensor has linear response range in this range.

2.10.5 Stability Stability of the sensor refers to its ability to function within the performance specifications under constant external conditions. There are several reasons for the failure of the sensor:

- Consumption of the reference electrode
- Depletion of the solvent medium
- Contamination of the working electrode surface
- Short-circuiting of the anode and cathode

Consumption of the reference electrode is a primary cause of failure of oxygen sensors, especially when it is used to carry current while it serves as a reference electrode (i.e., when used in the two-electrode mode). In the two-electrode mode, the current vs. resistance (IR) voltage developed across the resistive layers formed on the electrode may also lead to nonlinearity of the signal.

Another problem is contamination of the working electrode surface. For example, when a Ag/AgCl reference electrode is used, especially in a two-electrode mode, Ag can be transferred from the anode to cathode, possibly within hours of operation. In the extreme case, a silver bridge will be formed between the anode and the cathode, resulting in complete failure of the sensor.

Two steps could reduce this problem:

- Use a three-electrode system, so that no current flows between the anode and the cathode.
- Use an electrolyte composition with minimum silver solubility.

Another common reason for the wear out of the sensor is the loss of solvent. Methods to reduce this loss were discussed in section 2.8.

2.10.6 Flow Dependence The oxygen sensor, when placed in a stagnant liquid, produces a diffusion layer that could extend beyond the membrane. When the liquid is stirred, the diffusion gradient can no longer be extended beyond the stagnant liquid film very close to the membrane (fig. 30). The thickness of this liquid film very close to the membrane depends on the stirring or flow rate of the liquid. Because the diffusion gradient becomes steeper with decreasing thickness, the current increases with higher flow, which is called the flow dependence of the sensor.

One way to eliminate the flow dependence is to restrict the depletion layer within the hydrophobic membrane so that stirring will not affect this layer. This can be done by increasing the thickness of the membrane; however, this is at the expense of the response time. Another technique is to use a smaller cathode so that the reagent consumption is smaller; hence, the depletion layer is very small. For example, when the cathode diameter is less than 1 m, the probe becomes insensitive to liquid

flow even without the membrane [23]. Of course, as the diameter of the cathode becomes smaller, the total current output falls, and this may give rise to a problem of amplification.

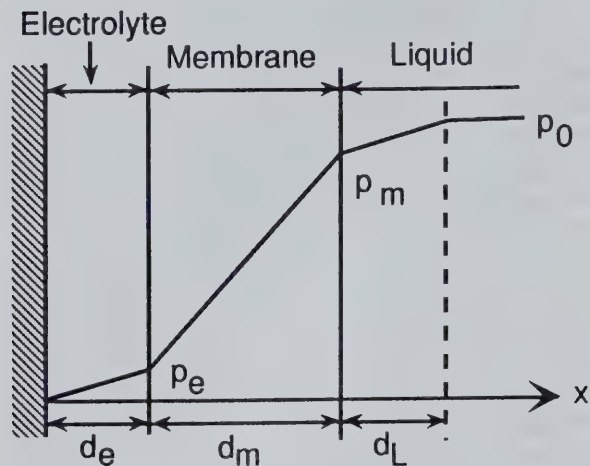


Figure 30. Concentration profile of oxygen in an oxygen sensor.

2.10.7 Temperature Effect When temperature causes negligible dimensional changes in the membranes, the only parameter of major importance that varies with temperature is the membranes material-permeability coefficient [24]. So the choice of membrane material is vital in designing the oxygen sensor. It is also very important that the membrane does not exhibit any change of phase over the operating temperature, which could change the permeation characteristics dramatically.

Relatively large signal changes are experienced with only small changes in membrane temperature. They range from 1% to 6%/°C depending on the membrane material utilized. So, in applications where rapid changes of temperature occur, temperature compensation of the signal prior to readout is mandatory.

2.10.8 Biocompatibility For in vivo applications, the sensor should be biocompatible. Apart from patient safety, the sensor could give faulty measurements if protein or other biological materials get deposited on the outer surface of the membrane. This deposit could behave as an additional membrane and also inhibit oxygen supply to the cathode. Frequent calibrations are required for contaminated probes. The contamination may be detected from the response of the probe, because all contaminated probes respond slowly.

2.10.9 Sterilization For in vivo use, the sensor should be sterilizable. So, how the sensor could be sterilized, packaged, and stored is very important.

Typical sterilization procedures involve autoclaving, gamma radiation, or ethylene oxide treatment. The effects of ethylene oxide residues on the chemical selectivity and sensitivity of the sensor should be determined as well as the question of whether sterilization by radiation will affect sensor performance. For example, gamma-sterilization could change the cross-linking of polymer materials, such as hydrogels, used in the fabrication of the sensor, changing the electrode performance. Several components of the sensor may not withstand the conventional steam-sterilization method.

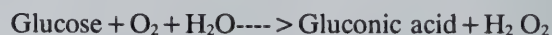
3. Glucose Sensor

3.1 Clinical Need

The detection, identification, and quantification of glucose in blood has played a vital role in the diagnosis and management of patients suffering from disorders of carbohydrate metabolism and is one of the most frequently performed determinations in clinical chemistry. In normal people, regulatory mechanisms operate so that blood-glucose concentrations are generally maintained within the range 2.5 to 7.3 mM. However, in diabetic patients, there is an elevation of glucose due to a relative or absolute deficiency of the hormone insulin. There are two forms of diabetes mellitus [2]. Type 1 occurs frequently in children. This form of diabetes is susceptible to ketoacidosis and, if not treated with insulin, usually results in death within 2 years. Type II, or maturity-onset diabetes, does not result in ketoacidosis. Both forms manifest a comparable number of secondary complications if careful treatment is not provided. These complications affect the eyes, kidneys, nerves, and blood vessels.

3.2 Theory and Operation

In its simplest form, an electrochemical glucose sensor consists of glucose oxidase enzyme held in close proximity to an electrochemical detector (fig. 31) that monitors the concentration of the reactants or products in the enzyme reaction.



In making a glucose sensor, either consumption of oxygen or production of hydrogen peroxide could be monitored amperometrically. Alternatively, pH change resulting from the reaction can be monitored potentiometrically. Potentiometric methods based on pH measurement are dependent

Enzymes

Dialysis Membrane

Currently, there are three categories of glucose sensors developed. Bench top glucose analyzers are used in hospital setting and are mostly based on the amperometric detection of H_2O_2 formed at a glucose oxidase membrane. A number of companies make such analyzers, the most prominent being the one from Yellow Springs Instruments Co., Yellow Springs, Ohio. The performance of the electrode is good and is used in routine clinical analysis in hospitals.

A third category is the implantable electrode for continuous glucose monitoring. Such a sensor, if developed, could be implanted in the body to adjust insulin dose for optimum insulin therapy. It is the most ambitious of the three categories of sensors and it has the most stringent requirements.

- Site: Ideally noninvasive
- Size shape: If invasive <20 G needle
- Materials: Biocompatible
- Range: 0–40 mM
- Stability: <10% drift per day
- Operational stability: Several months
- Specificity: No interference from other biospecies
- 95% response time: 30 seconds
- Calibration: No calibration preferred. If needed,
> every 12 hours
- Sterilization: Sterilizable
- Operation: Simple
- Cost: Inexpensive
- Sensor shelf time: At least 6 months

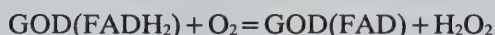
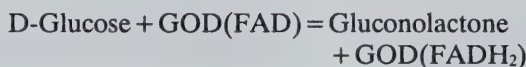
Implantable sensors have the most stringent requirements, and—although considerable progress has been made—the sensor has not been implemented for *in vivo* use for several reasons. One main reason is the interference from other species that are present in blood. Hydrogen peroxide produced from the enzyme reaction is monitored at +0.7 V with respect to a saturated calomel electrode. At this voltage, many other species present in blood—such as ascorbic acid (vitamin C), uric acid, bilirubin, and acetaminophen—are oxidized, contributing to an increased current.

The problem of oxygen deficiency has been attacked by a number of people from different angles. Gough et al. [25] proposed a novel electrode design in which oxygen can diffuse into the enzyme

layer across the open end and radially across the hydrophobic tube, but glucose has access only in the axial direction. With this design, the consequence of oxygen deficiency has been considerably reduced.

Other strategies reduce oxygen dependency by restricting the permeability of glucose to the electrode surface while at the same time allowing free diffusion of oxygen. This was achieved by coating electrodes with membranes: polyurethane [26], nylon, or cross-linked bovine serum albumin [27]. However, the most elegant method of avoiding deficiency, which also nearly eliminates the first problem of interference by enabling the application of a much lower voltage than used in the detection of hydrogen peroxide, is by the use of a mediator.

Oxygen dependency is eliminated by incorporating a redox mediator, which transfers electrons directly from the prosthetic group (FAD) of reduced glucose oxidase to a base electrode. The mechanism of the glucose reaction in the presence of oxygen and in the absence of an electron mediator is shown below:



where GOD(FAD) and GOD(FADH₂) represent the oxidized and reduced forms of the enzyme. If the reduced form of the enzyme can be oxidized electrochemically, the dependence on oxygen for the reaction can be reduced. However, the FAD fraction is buried well within the enzyme and is not readily accessible for oxidation.

Cass et al. [28] proposed the use of a low-molecular-weight mediator to shuttle electrons between the active redox center of the enzyme and the electrode (fig. 32).

The reagent should meet several criteria for its use as a mediator:

- Rapid electrode kinetics and rapid homogenous kinetics with the enzyme
- Unreactivity of the reduced form with oxygen
- Chemical stability
- Easy availability
- Low toxicity
- Redox potential comparable to that of the enzyme.

Ferrocene and its derivatives exhibit all the properties of a good mediator, and glucose sensors

based on this mediator have very low oxygen dependency over a wide range. In addition, because the electrode is operated at a very low potential (100–200 mV vs 700 mV for the H₂O₂ based detection), interference from electroactive species in blood is considerably reduced.

A glucose sensor based on ferrocene for in vivo application was developed by Claremont et al. [29]. In vitro, the sensor is linear up to > 20 mM and has a 90% response time of 68 seconds, and the current is independent of oxygen concentration in the normal range. In vivo studies using this sensor implanted subcutaneously in pigs indicates that the baseline tissue glucose is about 20% of the simultaneously measured blood-glucose concentration. However, the current output of the electrode closely followed changes in blood-glucose concentration.

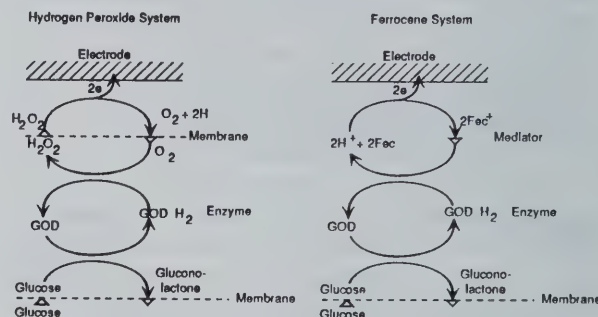


Figure 32. Glucose electrode chemistry with oxygen or ferrocene as mediators.

A second approach has been the modification of the enzyme by covalent attachment of redox mediator groups. Degani and Heller [30] reported the successful modification of glucose oxidase by the covalent coupling of ferrocene carboxylic acid to the enzyme using 1-(3-dimethylaminopropyl) -3-ethyl carbodimide hydrochloride. After most chemical modifications, the enzyme retains 25%–75% of its normal catalytic activity. The problem with this approach was that the stability of the modified enzyme was poor. Bartlett and Whittaker [31] have shown that ferrocene acetic acid is a better choice for the modification of glucose oxidase compared to carboxylic acid derivative.

A third alternative is the use of conducting salts as mediators or base electrode materials [32,33,34]. For example, the salt formed between tetracyanoquinodimethane (TCNQ) and tetrathiafulvalene (TTF + TCNQ⁻) is a good choice because of its stability and capacity to mediate the enzyme reaction. A glucose sensor based on TTF. TCNQ as

the electrode material has been successfully used to monitor glucose in a rat brain continuously for 28 days [35].

3.5 Problems with Commercialization

A reliable, clinically usable *in vivo* glucose sensor has not been developed yet. Although the experimental prototype sensors described before show promise, several problems have to be solved before they become successful products.

3.5.1 Electrode Stability A significant problem with most sensors constructed to date is drift in the output current when the external condition is kept constant. For example, figure 33 shows the response of a ferrocene-based sensor over a period of three days in solution.

Recent experiments [36] indicate that ferrocene-based glucose sensors can be reactivated by the addition of fresh glucose oxidase but not ferrocene. The sensor drift may hence be due to enzyme loss. Hence, better immobilization has to be worked out to prolong the life of the sensor.

3.5.2 Influence of Temperature Insulin-induced hypoglycemia is associated with a fall in both skin and core temperature of up to 2 °C, probably because of sweating, peripheral vasodilation, and inhibition of sweating [37,38]. An increase in body temperature during fever is a possibility too. Temperature has a significant influence on the response of the sensor. The effect of temperature could arise from several factors. The diffusion of the substrate could be considerably changed by difference in temperature. Also, the response of the electrochemical detector is influenced by temperature. Temperature coefficients of 3%–6% °C is not unusual with ferrocene-based glucose sensors, and the temperature of the sample should be carefully controlled or compensated for to obtain best results. On the other hand, small inaccuracies in glucose measurement may not be of clinical significance that variation in body temperature will probably not invalidate the results.

3.5.3 Toxicity and Biocompatibility The inclusion of new materials such as mediators in the sensor fabrication raises the question of toxicity. Ferrocene has been suggested as a possible haematinic agent; however, no evidence yet indicates that its use in humans at reasonable doses produces any adverse effects. No such data are available for TTF.TCNQ or a number of other mediator materials used.

A second problem is the deposition of fibrinous proteins on the sensing element or encapsulation

by cells that impose an additional diffusion barrier for glucose, which will result in a decrease in sensor output. The use of a material with a high degree of biocompatibility may look like an obvious solution, but the membrane may not be compatible with the function of the sensor.

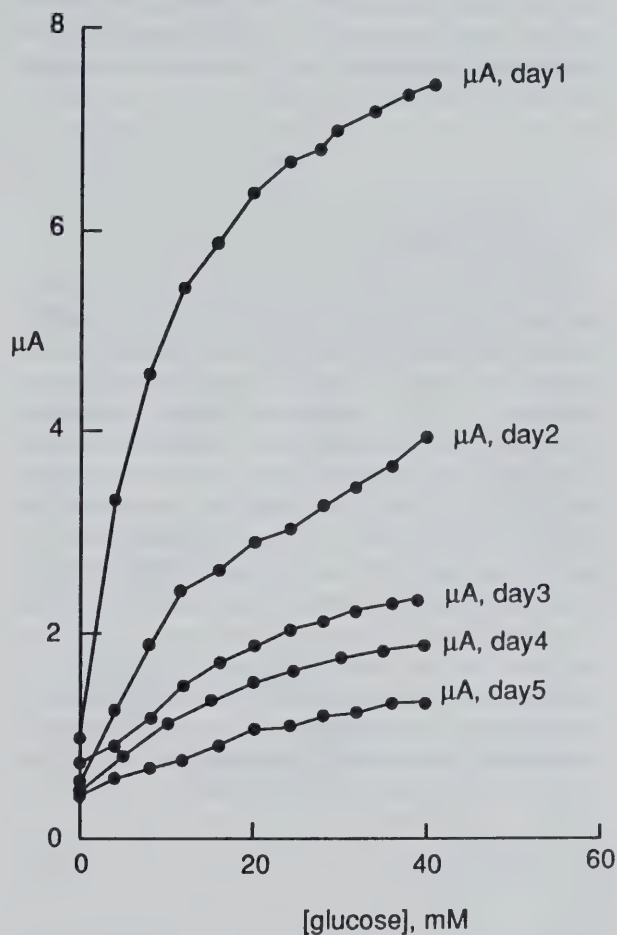


Figure 33. Stability data for a glucose sensor based on ferrocene mediator.

3.5.4 Shelflife and Calibration These are two important points that must be taken care of before a commercially successful *in vivo* glucose sensor can be realized. At present, there is no information on how to store sensors or how they deteriorate over a period of storage.

If *in vivo* glucose sensors are to make a major impact on the management of diabetes, they must be easy to use. It is not known that an enzyme sensor could be factory calibrated and remain stable for several weeks before it is used. However, the fact that the commercially available *in vitro* sensor is not calibrated before use, points to the possibility

of a factory calibration. If the patient has to calibrate the the sensor prior to use, relatively sophisticated instruments will be required and will restrict the widespread use of such sensors.

3.5.5 Sterilization The sterilization of glucose sensors has problems similar to those discussed for oxygen sensors. In addition, because an enzyme is used in the construction of the sensor, one of the critical requirements of the sterilization method is that the method should not alter the biological activity of the protein.

4. Urea

4.1 Clinical Need

The determination of urea nitrogen is presently the most widely carried out screening test for the evaluation of kidney function. In addition, the follow-up of urea kinetics is the only objective parameter for the estimation of dialysis efficiency. The main disadvantage of the current method in checking the dialysis efficiency is that it is performed at intervals, and—because of a certain degree of complexity—it is only performed in specialized units. The current method does not allow a continuous control of the results obtained. Continuous measurement of urea would avoid inadequate dialysis, resulting in increased toxicity, as well as overdialysis, which increases the risks of dialysis-related complications. In other words, with continuous monitoring, each dialysis could be adapted to the needs of the patients.

4.2 Requirements

Range: 3×10^{-4} – 2×10^{-2} M

Response time: <1 min.

Baseline recovery: <2 min.

Stability: 2% per 6 hours

4.3 Current Status

The first urea electrode was prepared by Guilbault and Montalvo [39] by immobilizing urease on an ammonium-selective glass electrode. The urea diffuses to the urease membrane, where it is hydrolyzed according to the reaction, and the NH_4^+ ion is monitored by the ammonium ion-selective electrode. The potential observed is proportional to log (urea) concentration in the range 1.0–30 mg of urea/100 mL (1.7×10^{-4} – 5.0×10^{-3} M) of solution. The selectivity of commercially available glass

electrodes is so poor that they cannot be used in the presence of physiological levels of K^+ and Na^+ .

To eliminate interferences from Na^+ and K^+ ions, Guilbault and Hrabankova [40] used an uncoated NH_4^+ ion electrode as reference electrode to the urease-coated NH_4^+ electrode. However, the interference from these ions is not completely eliminated, so an ion-exchange resin had to be used in conjunction for assay of blood and urine.

In attempts to improve the selectivity of the urea determination, Guilbault and Nagy [41] used a silicon rubber-based nonactin ammonium selective electrode as the base sensor, together with immobilized urease in polyacrylic gel. The ammonium selective electrode made using the antibiotic nonactin as the active ingredient has very good stability and better selectivity. The selectivity coefficients of the electrode were 6.5 for NH_4^+/K^+ , 7500 for $\text{NH}_4^+/\text{Na}^+$, and much higher for other cations. Even with the new NH_4^+ ion electrode, sufficient selectivity is not reached to eliminate the effect of K^+ . Even better results could be obtained if the measurements are made versus an ammonium ion selective electrode [42]. Thus interference from K^+ is almost eliminated. No interference from Na^+ in the physiological range is observed because of the high selectivity ratio.

The urea sensor could also be based on either NH_3 or CO_2 gas measurement. Anfalt, et al. [43] used a commercially available ammonia gas sensor to construct a urea sensor. While gas sensing will have least interference from ionic components present in the sample even if the sample is as complex as blood, the amount of NH_3 or CO_2 present at the physiological pH of 7.4 is only a minor fraction of the total amount of these gases produced from the enzyme reaction and the measured signal will be low. The response of the urea sensor based on gas sensing was slow (2–4) min, and the recovery was 10 min or more. A more quickly responding urea sensor (response time of 30–45 seconds with a much faster baseline recovery than that described before) was developed by Joseph [44].

Alternatively, a pH sensor to measure the pH change due to products formed could be utilized. Nilsson et al. [45] have developed a urea sensor by immobilizing urease on a glass electrode. The response time of the electrode to urea was about 7–10 min and a linear range of 5×10^{-5} to 10^{-2} M was obtained. Because glass electrodes are fragile and their miniaturization is difficult, Alexander and Joseph [46] replaced glass pH electrode with an antimony pH sensor to construct a urea sensor.

The urea sensor gave a linear response to urea in the range of 5×10^{-4} to 10^{-2} M, and its response was much faster. Later, Joseph [47] improved the immobilization method to increase the lifetime of the electrode from 1 week to about 3 months.

Because the response of the urea sensor based on pH measurement is affected by the pH variation from the sample—not from the enzyme reaction—a differential measurement should be used. The response of the urea sensor is measured with respect to another pH sensor not coated with the enzyme. This will eliminate errors resulting from any difference in pH of the sample before the enzyme reaction is initiated.

Antimony pH sensor has been known to have unreliable potential and drift and to be influenced by redox materials present in the sample, including oxygen. For a metal-oxide electrode to be useful as a pH sensor, it should satisfy several important criteria. For example, the metal must be noble enough to resist attack by solutions over a useful pH range (for example, for urea the electrode should be between pH 6 and 9), and its oxide must come into equilibrium with the solution without appreciable dissolution. As these basic requirements are somewhat difficult to achieve, most of the metal-oxide electrodes, including antimony electrodes, used for pH indication represent a trade-off between the nobility of the metal and the stability of the oxide.

A metal-oxide electrode that has been recently investigated is iridium oxide. Iridium oxide electrodes give a near-theoretical response to pH over ranges of pH 0–14 [48]. Iridium oxide electrodes can be prepared thermally, electrochemically, or by sputtering. It has been observed that iridium oxide prepared by sputtering has much less redox sensitivity, although in our experience ascorbic acid still causes some interference [49].

Teknekron Sensor Development Corporation (TSDC) has constructed a urea sensor based on iridium oxide electrode, and it gives excellent characteristics in samples containing no ascorbic acid. Ascorbic acid even at levels of 0.1 mg/dL of sample causes a shift of electrode potential and is an interference [49]. TSDC is experimenting with methods of preparing of iridium oxide and using membranes to avoid interference problems.

5. Cholesterol

5.1 Clinical Need

Coronary heart disease, caused usually by arteriosclerosis (a term referring to a number of pro-

cesses, all of which produce thickening of the blood-vessel wall), is the number one killer disease in the United States today, responsible for over 500,000 deaths annually. High serum cholesterol has been established as one of the major risk factors for the disease.

A large number of studies have resulted in convincing evidence of the strong and direct relationship between the concentration of serum cholesterol (especially the low-density lipoprotein-cholesterol fraction that normally holds 60% to 75% of the total cholesterol in serum) and the rate of development of arteriosclerosis and coronary heart disease morbidity and mortality [50]. Also, studies have shown that progression of severe arteriosclerotic lesions can be retarded, prevented, and/or reversed in the rhesus monkey and that healing of the damaged arterial endothelium can be achieved by substantial lowering of blood lipids after a relatively short period of low-fat, low-cholesterol diet and/or drugs.

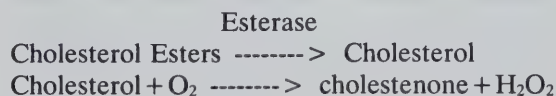
There is need for an assay that can be performed rapidly, accurately, and at low cost by persons such as general medical practitioners in their surgery. While the ultimate goal of screening is to get the complete lipid profile, analysis of total serum cholesterol can be the initial step of evaluation.

5.2 Availability of Sensors

Reportedly, several companies are working on a total cholesterol sensor. However, no product has been realized yet. Total lipid profile is based on instrumental methods only, and—to my knowledge—nobody is working on a sensor to do the same function.

5.3 Principle

The measurement of cholesterol is based on the enzyme reaction.



Clark et al. [51] described an electrode for total cholesterol based on measurement of H_2O_2 using a Pt electrode. The enzyme cholesterol oxidase was immobilized on the electrode and esterase was used as soluble form. Linearity was obtained over the range of 25 to 300 mg cholesterol/100 mL, with a coefficient of variation of 4%. However, H_2O_2 detection cannot be used in blood samples because of

interference from oxidizable species (ascorbate, urate, tyrosine, etc.).

Coulet and coworkers [52] described an enzyme electrode using collagen-immobilized cholesterol oxidase for the determination of free cholesterol. A linearity from 10^{-7} M to 0.8×10^{-4} M (.004 to 3 mg/100mL) was obtained. However, a nonenzymatic electrode had to be used to correct electrochemical interferences.

Dietschy [53] and Noma and Nakayama [54] reported cholesterol sensors by measuring dissolved oxygen consumed in the reaction media during the enzymatic reaction. They obtained a linear relationship between oxygen consumption and cholesterol concentration up to 8 g/L. Bilirubin and ascorbic acid did not interfere. However, the method is sensitive to oxygen-concentration variations. In addition, because of the oxygen requirement of the reaction, sample dilution is necessary to overcome oxygen depletion.

Similar to the glucose sensor, interference to the cholesterol assay using electrochemical means could be eliminated by the use of a mediator. This is based on the ability of ferrocene to act as an electron donor. The analytical system exploits the ability of ferrocene derivatives to mediate electron transfer between peroxidase enzyme and the electrode. The reaction is the two-electron oxidation of peroxidase in the presence of H_2O_2 produced from the cholesterol reaction, H_2O_2 itself being reduced to water. The native enzyme is regenerated by electron transfer from a suitable mediator in its reduced form.

6. Conclusion

Sensors are a rapidly expanding field of technology. Uncountable numbers of difficult technical problems have been solved over the years by dedicated scientists and engineers. However, among the sensors discussed, only one sensor for glucose that is suitable for in vitro, discrete analysis is commercially available. Several reasons could be cited for this delay in commercialization.

Most of the work in the biosensor technology is at present carried out in single-discipline research laboratories. Sensor research is a multidisciplinary area, and it is necessary to put together such a team in one building. The transition from laboratory to mass production involves a number of major manufacturing steps. Batch processes have to be developed for reproducible manufacture of the base electrode, deposition of the mediator, immo-

bilization of the enzyme, encapsulation of inactive areas of the sensor, packaging, and sterilization. The reproducibility from sensor to sensor within one batch should be such that a factory calibration of a few sensors from a batch should be representative of the whole batch within a narrow pre-set value. All these can only be achieved by a substantial financial input and dedication.

Most biosensor applications require the product to have a shelflife of several months, and the majority of biocomponents do not have sufficient storage stability. So stability has to be improved by better immobilization procedures. Also, the biological activity of such components could be lost or considerably reduced during typical deposition procedures.

In short, several problems have to be solved before a sensor becomes a success. However, the development of new technologies has always posed challenges, especially in the clinical diagnostic area. The problems posed by the introduction of one type of sensor, the ion-selective electrode, are now being addressed, and the sensors offer a convenient method of analysis for a number of ions.

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7. References

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Biological Applications of Electrolyte/Insulator/Silicon Structures

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Starting with the earliest work in the field, many of the applications of chemical sensors in silicon have been biomedical or biological. This chapter discusses the biological applications of one particular type of silicon chemical sensor: the Electrolyte/Insulator/Silicon (EIS) structure.

The best known type of EIS sensor is the ISFET or CHEMFET, and much work has been published about those devices. Recently another type has been described, the Light Addressable Potentiometric Sensor (LAPS). The advantages of LAPS devices are discussed.

The first family of devices with biological applications are those involving immobilized enzymes. An overview is given of the various methods used to immobilize enzymes in a thin film over an electrode. The ENFET is a device in which such a film is placed over the gate of an ISFET. Its sensitivity depends on mem-

brane thickness and buffer capacity. Another type of measurement system uses a LAPS and a microvolume chamber. By reducing the solution buffer capacity and the chamber volume very high sensitivity can be achieved in a sandwich immunoassay format, in which the enzyme is used as a detection label.

Another family of work involves the use of EIS structures to measure the metabolic rates of living cells. These measurements have applications such as in vitro toxicology, study of receptor/ligand interactions, study of chemotherapeutic and antiviral drugs, and medium optimization.

Key words: biosensors; CHEMFET; ENFET; immobilized; immunoassay; ISFET; LAPS; metabolic rate; receptor/ligand interactions; silicon chemical sensors.

1. Introduction

The original concept of silicon devices sensitive to chemical quantities was born of the analogy between the silicon dioxide, which is used as the gate insulator in metal oxide semiconductor field effect transistor (MOSFET) transistors, and the glass in electrodes, which are used for pH measurement. The very high impedance of these glass electrodes requires an amplifier with high input impedance, which typically means a MOSFET input stage. Bergveld was the first to recognize that because the gate insulator of a MOSFET is silicon dioxide, a material similar to the glass of a pH electrode, it should be possible to eliminate the metal gate of the MOSFET and directly use the gate insulator as

a pH-sensing material [1,2]. Upon immersing a MOSFET without the metal gate in solution, it was found that the threshold voltage of the transistor indeed depended on the solution pH. Bergveld called this device an ion-sensitive field effect transistor (ISFET). It was one of the first silicon devices to be used as a sensor, and it served as an example for many more to follow.

It took some time for the concept of using an FET transistor as a chemical sensor to become accepted. Only a few researchers worked in this field between 1970, the year of Bergveld's first paper, and 1975. In the electronics community, the usual reaction to the ISFET concept was that after so

much effort had been expended to fabricate an FET transistor in a totally sodium-free environment, it would be fatal to expose such a transistor to sodium-containing solutions. That objection proved to be unfounded, however, because sodium is only mobile in silicon dioxide at temperatures well above room temperature. Since 1975, the amount of work done on silicon sensors has steadily increased, and a broad variety of types of devices have been receiving attention. Devices now exist for sensing physical quantities such as pressure, acceleration, flow, and magnetic fields. Also in existence are gas-phase chemical sensors and liquid-phase chemical sensors for ions, enzyme substrates, and antigens. Several good broad overviews of solid-state sensors have been published [3,4], and they should be consulted to appreciate the breadth of work done in this area.

In the field of chemical sensing in liquid environments, several new types of devices have been proposed based on electrolyte/insulator/silicon structures (EIS). This is the basic structure used in an FET, but simpler versions can also be used as sensing devices. Thus, it is appropriate to broaden the area that will be covered in this survey to EIS structures in general. It was quickly realized that these sensors would be most useful in biological and medical applications, because that is where the advantages of small size, in-situ impedance transformation, and robustness were of most value. A good early example is the use of an ISFET as a tool for recording biopotentials [2], where the impedance transformation at the point of the recording is very helpful in reducing noise. In analytical chemistry, or process engineering, there are fewer compelling reasons to replace conventional glass electrodes or ion-sensitive electrodes.

One of the areas in which much work was done was in the use of the ISFET structure as a starting point for more complex systems, involving membranes or other transducer elements on top of the gate insulator. Another area in which complexity was added was the integration of the sensor in a measurement system that includes fluidics to manipulate the sample. A good example of this is the use of a sensor as the detector of a flow-injection analysis system [5]. Finally, more and more attention is being devoted to biological applications. Much work in that field involves the use of immobilized enzymes, a topic that is covered here. Some of the most difficult aims in biology have been the attempts to detect antigen-antibody binding with a potentiometric sensor such as an ISFET. Recently, some approaches to this problem have been de-

scribed. Finally, some of the most recent work to be described on biosensors with EIS structures involves the use of living cells.

The scope of this text will include devices based on EIS structures, which are all potentiometric, and the systems in which they can be used in biology. There exists a whole family of amperometric biochemical sensors, typically using immobilized enzymes to detect enzyme substrates, which will be covered elsewhere. After dealing with the basic description and properties of EIS structures, the next two sections will deal with biosensors using enzymes and biosensors using whole cells, respectively.

2. Electrolyte/Insulator/Silicon (EIS) Structures as Chemical Sensors

Electrolyte/insulator/silicon (EIS) structures comprise a broad family of potentiometric silicon sensors. The best-known member of the family is the ion-sensitive field effect transistor, known as the ISFET or CHEMFET. Sensing transistors of this type have applications beyond the domain of chemical measurements in liquids; they can also be used as gas-sensing devices (palladium-gate FETs, for instance) or in general to detect any quantity that can be transformed into an electrical potential difference by a layer present on top of the insulator layer. Recently, another type of EIS sensor has been invented by Hafeman et al. [6] the LAPS, or light-addressable potentiometric sensor. Its advantages will be described below (sec. 2.1.4).

The differences between the different types of EIS sensors are mostly an issue of the fabrication process and the type of electronic circuitry and software needed to obtain measurement data. The principles behind the operation of all these devices are the same. Therefore, I will treat all EIS sensors together as one family, except in a few brief sections (2.1.3 to 2.1.5) where I cover the particularities of the individual members of the family.

2.1 Basic Analysis

2.1.1 Description of an EIS Structure A basic diagram of an EIS structure is shown in figure 34. The semiconductor used is almost always silicon, although other semiconductors are certainly also possible. The usual choice of silicon is merely a matter of convenience. The basic principle of operation of devices using such structures is as follows. A potential is generated at the interface between

the liquid solution and the insulator. If there is no additional potential-generating layer present, then a pH-dependent potential is generated, as described below. If one desires a sensor for a quantity other than pH, then an intermediary layer to generate a potential sensitive to that quantity is needed. Examples of such layers are PVC/plasticizer membranes containing ionophores such as valinomycin or nonactin (for devices sensitive to K^+ or NH_4^+) or gold to obtain a device sensitive to redox potential. For a given potential applied between the solution and the silicon substrate, the potential between the solution and the insulator will affect the electric field in the insulator. This electric field in turn affects the surface potential of the silicon. This surface potential in turn changes a number of properties of the silicon surface, which can be measured in different ways, depending on the type of EIS structure.

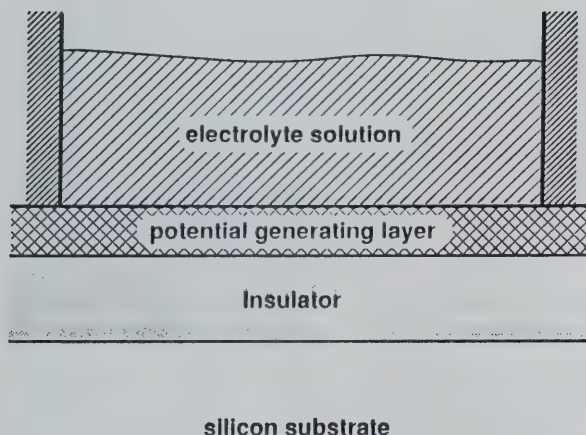


Figure 34. Basic diagram of an EIS structure.

The measurement typically requires adjusting or sweeping the potential between the solution and the silicon to determine or maintain a property of the silicon surface. For an ISFET, that property would be the lateral conductivity between two oppositely doped regions (the source and the drain) surrounding the active area. For an EIS capacitor, it is the capacity of the structure, or the flat-band voltage. For a LAPS, it is the inflexion point of the photocurrent-voltage curve. In all cases, the presence of a semiconductor surface enables the detection of variations of the electric field in the insulator. This is what enables the measurement of changes of potential at an interface between an insulator and an electrolyte, even though the insula-

tor makes a conventional direct-potentiometric measurement impossible.

It is important to remember that from an electrochemical point of view, an EIS structure is only one electrode in a cell. In order to set the potential of the solution, another electrode is needed—typically a reference electrode. The EIS structure, together with the reference electrode in the solution, can be seen from two points of view:

1. From the electrochemical point of view, this is an electrochemical cell with two electrodes. The particularity of the cell is that it does not itself generate a measurable voltage, as is the case with most cells. This is due to the very high impedance of the insulator in the EIS. To make a measurement, a voltage is applied to the cell, and a property of the silicon surface is measured. The effective cell voltage is then the potential that must be applied to it to produce a given reference situation such as a flat-band voltage or a threshold voltage. Because the reference electrode is designed to have a constant potential, the cell voltage varies only with the potential at the insulator/electrolyte interface.
2. From the silicon-device physics point of view, the structure of figure 34 is a field-effect device where the gate is provided by the reference electrode and the electrolyte, instead of by a metal or by polysilicon. The gate lead contains a number of potentials in series—namely, the externally applied potential, the constant potential between the reference electrode and the solution, and the potential at the insulator/electrolyte interface. The variation of this last potential due to chemical changes has exactly the same effect as the variation of the externally applied potential. The equivalent circuit description of the insulator/electrolyte interface is a chemically dependent battery in series with the insulator.

EIS structures have another very important characteristic—namely, the decoupling of the behavior of the electrical double-layer at the insulator/electrolyte interface and the electric fields and charges in the silicon surface and in the insulator. In effect, the charge and potential in the double layer only depend on the ions in solution—most notably the pH. From the point of view of the electrical equivalent circuit of the device, the potential in the double layer acts as a battery in series with the gate capacitance. The reason for this decoupling is that the charges, electric fields, and capacitances in the

double layer are much higher than those elsewhere in the structure. A typical double-layer capacitance is $20 \mu\text{F}/\text{cm}^2$. This means a potential of 100 mV, a typical order of magnitude, causes a charge of $2 \mu\text{C}/\text{cm}^2$, or 1.2×10^{13} charges/ cm^2 . This is about two orders of magnitude greater than the charge in a silicon surface at the onset of strong inversion. This charge, in a layer of oriented water dipoles with a relative dielectric constant of 6, will induce an electric field of about 4×10^6 V/cm. This is again much higher than would be encountered in the insulator during the operation of an EIS device. The conclusion is that the charges and fields required in an EIS device are too small to affect the double layer, which thus remains controlled by the solution pH.

2.1.2 Flat-Band Voltage of an EIS Structure

The basic quantity measurable in an EIS structure is its flat-band voltage V_{FB} . This is the quantity that will vary as a result of changes in the electrolyte solution. The analysis of V_{FB} is similar to that of the flat-band voltage of an MIS (metal/insulator/semiconductor) structure, which is well-known in the literature of electronic devices. The difference is that the metal gate is replaced by a reference electrode immersed in a solution. The flat-band voltage of an EIS structure has been shown [7] to be given by:

$$V_{\text{FB}} = E_{\text{ref}} - \frac{\Phi^{\text{Si}}}{q} - \psi_0 - \frac{Q_{\text{ins}}}{C_{\text{ins}}} + \delta_x \quad (1)$$

where V_{FB} is the flat-band voltage of the structure, E_{ref} is the reference electrode potential, Φ^{Si} is the work function of silicon, ψ_0 is the surface potential at the insulator/electrolyte interface, Q_{ins} is the charge at the insulator/silicon interface, C_{ins} is the insulator capacitance, and δ_x collects a number of dipole potentials which can be neglected (for a detailed discussion of dipole potentials, see reference [7]). The reference electrode potential E_{ref} is an "absolute" potential, i.e., relative to vacuum at infinity, as are work functions. There has been much discussion in electrochemistry of the meaning of such electrode potentials; currently there is agreement by a number of authors that the potential of the normal hydrogen electrode relative to vacuum is

$$E_{\text{NHE}} = 4.75 \text{ V} \quad (2)$$

There is some theoretical and experimental uncertainty concerning this value, and authors such as Trasatti [8] prefer the value $E_{\text{NHE}} = 4.44$ V. In my opinion, however, the coincidence of values found by various different techniques indicates that the

potential given in eq (2) is reliable to within 0.05 to 0.1 V.

It is generally accepted that the term in the flat-band voltage which is pH-sensitive is the surface potential ψ_0 (we will return to this subject in sec. 2.2). Unfortunately, the other terms in eq (1) are not known with a precision greater than a few hundred millivolts. This is true for E_{ref} , as noted above, but also for $Q_{\text{ins}}/C_{\text{ins}}$, which can vary from device to device by several hundred millivolts. For a given EIS sensor, these inaccurately known quantities are constant, and variations in flat-band voltage can be equated to variations of the surface potential. But absolute knowledge of the surface potential is not possible.

In particular, this means that an EIS sensor cannot measure directly the pH of zero charge, pH_{pzc} , of an insulator surface. A complementary quantity whose absolute value can be measured is the zeta potential, which arises when the liquid phase moves relative to the solid phase at an insulator/electrolyte interface. The total surface potential ψ_0 is the relevant quantity when using the device as a pH sensor, and closely follows Nernst's law for many insulators. But only variations of ψ_0 can be measured, not its absolute value. The zeta potential is lower than the total potential, and its definition involves the slipping plane whose location is not known exactly, but it can be determined absolutely. The ability to measure both for the same insulator films would provide an opportunity for more complete understanding of the chemistry of the surface. I am presently working on measurements of zeta potentials on insulator films used in EIS structures, and I expect to be able to report results soon.

2.1.3 EIS Capacitors The simplest type of EIS structure is the EIS capacitor. Figure 35 shows a schematic diagram of such a device. Measurements are taken by measuring capacitance-voltage (CV) curves. The theory of the capacitance-voltage curves of MOS devices is very well known [9]. It has been shown that the impedance of the insulator/electrolyte interface itself is almost always negligible compared to that of the rest of the structure [10]. Thus, all the usual methods for interpreting capacitance-voltage curves of MOS structures can be applied to EIS capacitors. Using these methods, either the flat-band voltage is determined directly from CV curves, or else the shift in V_{FB} is found from comparing the curves to a reference.

This device has had a relatively limited popularity; its main advantage is simplicity and the fact that it allows EIS structures to be characterized

without the complexity of a MOS-type fabrication process. The disadvantage is that the measurements yield curves to be interpreted, not numbers. The device is not easy to turn into a sensor with an output that is easy to use, although a feedback circuit for this purpose has recently been described [11]. Accordingly, most use of EIS capacitors has been for purely research purposes. In particular, it has been as a research tool to characterize the insulator/electrolyte double layer [12,13].

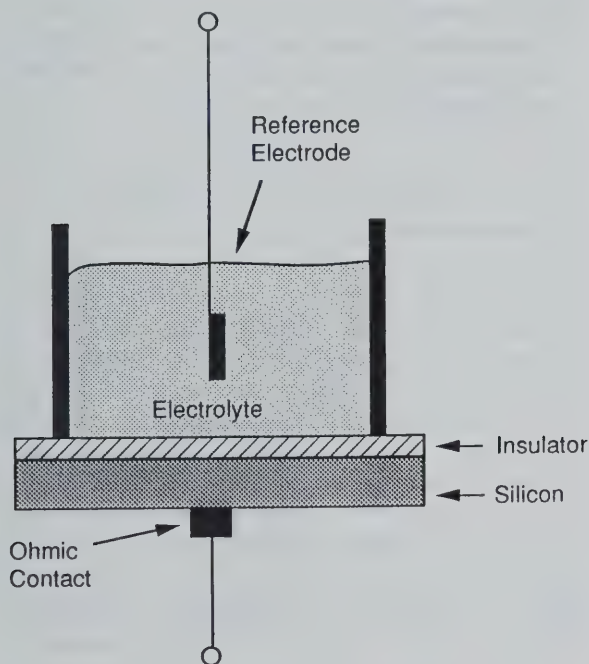


Figure 35. Schematic diagram of an EIS capacitor.

An important practical aspect of these measurements is the edge of the encapsulation of the solution. In an EIS capacitor, that encapsulation is inevitably part of the measurement. Typical methods to encapsulate the edges are rubber gaskets under pressure, epoxy adhesives, or silicone rubbers. In all cases, a thin film of liquid can seep under part of the encapsulation, either initially or after a certain length of exposure. As a result, the capacitance measured depends on frequency and increases with time. This can cause serious practical problems; possible solutions are the use of a field oxide to reduce the capacitance of the regions close to the edge of the device [10] or better encapsulants, which exhibit these problems only to a minimal extent.

2.1.4 EIS Structures Addressed by Modulated Light (LAPS) In 1988, Hafeman, Parce, and McConnell introduced a new sensing device based on an EIS structure [6]: a light-addressable potentiometric sensor. A diagram of the device is shown in figure 36.

In 1988, Hafeman, Parce, and McConnell introduced a new sensing device based on an EIS structure [6]. They called it a light-addressable potentiometric sensor, with the acronym LAPS. A diagram of the device is shown in figure 36.

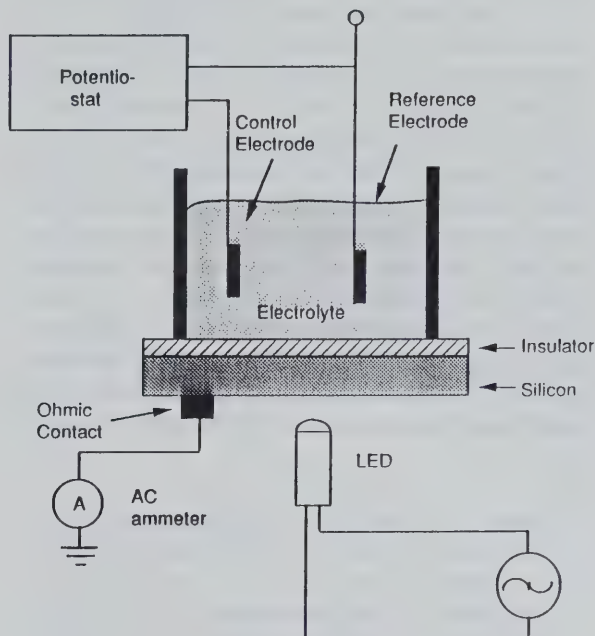


Figure 36. Schematic diagram of a LAPS device.

The operation of the device can be explained as follows. It is constructed exactly like an EIS capacitor as described in the previous section, with the addition of an ac modulated light source. Let's assume a dc voltage is applied that biases the structure in depletion or inversion. The light shining on the silicon substrate generates electron/hole pairs, which will be separated by the electric field in the depletion layer then present at the Si/SiO₂ interface. The minority carriers will drift to the surface, where they join the inversion layer. The majority carriers drift to the bulk. As a result of the added minority carriers at the surface, the silicon surface potential changes, and therefore a photovoltage is generated. By ac modulating the light, an ac photovoltage is generated that will cause an ac photocurrent to flow in the external circuit. When the applied dc voltage biases the structure at flat-band,

or in accumulation, there is no electric field in the silicon near the surface, and therefore no separation of the photogenerated carriers. At those biases there is therefore no photovoltage or photocurrent. Figure 37 shows an example of a curve of the ac photocurrent as a function of bias voltage. It is seen that there is a sharp transition between accumulation, where the photocurrent is zero, and inversion, where it is maximal.

To monitor changes in the potential at the insulator/electrolyte interface, the shifts of this curve along the voltage axis are monitored. This involves tracking the inflexion point of the curve—i.e., the point where the second derivative of the curve goes through zero—and where the first derivative is maximal (see fig. 37). This tracking method has the advantage of being independent of changes in amplitude or zero offset in the measurement of the photocurrent. The inflexion point voltage depends on the flat-band voltage of an EIS structure. Thus, the LAPS is another method of monitoring variations in V_{FB} .

The fabrication process to make a LAPS is relatively straightforward. Starting with low-doped (typically 15–30 $\Omega\text{-cm}$) n-type (100) silicon, the main process steps are (in the case where a field oxide is used):

1. Growth of a thick (1 μm) field oxide.
2. Patterning to remove the field oxide in the active areas.
3. Growth of a 30 nm gate oxide.
4. LPCVD deposition of a 100 nm layer of Si_3N_4 over the whole wafer.
5. Removal of the oxide and nitride on the back side of the wafer.
6. Deposition, patterning, and alloying of a metal on the backside for the substrate contact.

The LAPS has the following important practical advantages:

1. The fabrication process is much simpler than for ISFET structures. In its simplest form (without field oxide), no lithography is required.
2. The photocurrent/voltage curves are much steeper than capacitance/voltage curves, making it possible to track variations of surface potential with much less noise. In optimal conditions, noise is around 0.1 mV peak-to-peak.
3. The active area is defined by the extent of the light beam. This makes encapsulating the edges of the device much less critical than for an EIS

capacitor, because the edges are not part of the measurement.

4. The only contact to the chip is on the back side and therefore easy to protect from the solution. There are no bonding wires on the front side to encapsulate.
5. By multiplexing different light sources in different spots, the device can be a multisensor without additional process complexity. Also, the zone covered by one light source can be split up into many individual small sensing areas without added complexity.
6. The absence of front-side contacts or metalization means the sensor surface can be very flat and smooth.

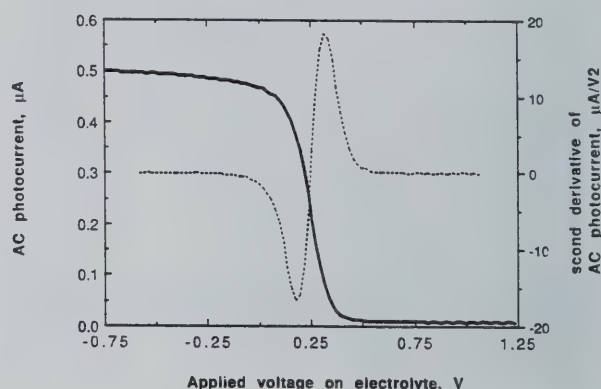


Figure 37. The ac photocurrent in a LAPS as a function of bias voltage.

The ability to fabricate multisensor structures without added complexity in the fabrication process is especially important. It will be discussed below that many applications of EIS structures appear to involve multiple sensing sites and combination with the immobilization of enzymes or cells in a small volume on top of the sensing surface. The LAPS is especially suited for such applications, and that characteristic will probably lead to its increasing popularity in the coming years.

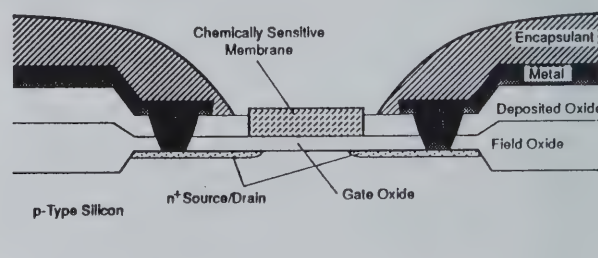


Figure 38. Schematic diagram of an ISFET.

2.1.5 EIS Structures as Part of Transistors: CHEMFETs The final type of EIS structure is the ion-sensitive field-effect transistor, known as ISFET or CHEMFET (see fig. 38). In this case, the surface potential is monitored by measuring the surface conductivity between two lateral regions doped to the type opposite of that of the bulk. These regions are known as the source and the drain. These devices behave electrically completely as field effect transistors, but their threshold voltage depends on the potential ψ_0 . This dependence is described in the same way as in the flat-band voltage, which was treated in section 2.1.2. The best way to think of an ISFET is shown in figure 39: it is as a conventional MOSFET with a pH dependent voltage supply in series with the gate. The reason that this is a valid representation is that the potential at the insulator/electrolyte interface depends almost entirely on the solution pH and very little on the applied voltage, as discussed in section 2.1.1.

ISFETs are the devices that most researchers have worked on. They can be small, and changes in their threshold voltage can be monitored by placing them in a feedback circuit [14]. These circuits typically produce output signals with a noise level of about 0.1 mV—similar to what can be obtained with LAPS structures. The ISFET itself acts as an active device and produces low-impedance outputs when placed in an appropriate circuit. Some of this circuitry can be placed on-chip [15,16].

Because they were the first chemical sensors on silicon to be invented, as early as 1970, there is more literature on ISFETs than on any other type of chemical sensor. This includes several reviews [17,18,19] and, more recently, books [20,21,22]. I will therefore not go more into the details of ISFET technology, because interested readers can refer to the given references for more information. Another reason not to dwell too much on this particular topic is that the commercial prospect of ISFETs by themselves appear bleak. Several ventures have tried unsuccessfully to produce these devices for sale; some of the problems encountered are lack of sufficient competitive advantage compared to conventional ion-selective electrodes, biocompatibility and blood clotting problems encountered during tests of short-term in vivo use, and high fabrication cost at low volumes due to the expensive integrated-circuit technology involved.

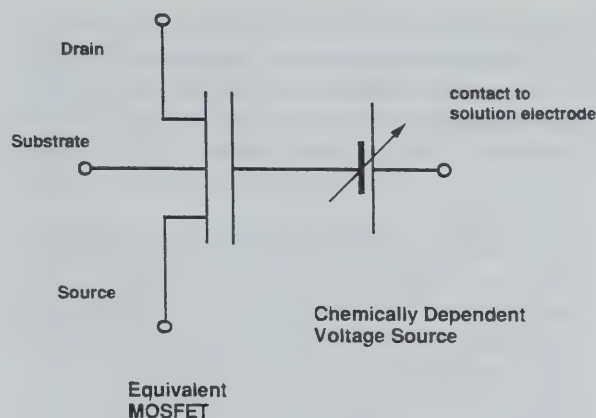


Figure 39. Electrical equivalent circuit of an ISFET.

Some of the drawbacks of FET-based devices are as follows. First of all, the fabrications steps required for an MOS process are considerably more complex and expensive than for a LAPS. Next, the source and drain regions require leads to the front side of the chip. These leads have to carry voltages, which makes it much more difficult to protect them from corrosion. Thus, the encapsulation problem here is much more difficult than for a LAPS device. Finally, FET devices do not lend themselves to easy multiplexing: at least one lead is needed per new device. Each of these leads again carries a voltage and is complicated to protect. A possible solution to this last problem is to use interconnects resistant to high temperatures, so that much of the circuitry on the chip, including the multiplexing, can be protected by a LPCVD silicon nitride layer [16]. But even then, at the edge of the chip, some signals and power supply leads have to go to the bonding pads, and that is the vulnerable location for reliability.

2.2 The pH Sensitivity of EIS Structures

2.2.1 Overview and Comparison With Glass Electrodes The origin of the pH sensitivity of a glass electrode is not an entirely closed subject. Original approaches on the topic centered attention on ion transport across the glass membrane. More recent work ascribes the pH sensitivity to the glass surfaces where large numbers of Si-OH (silanol) sites exist [23]. The glass surfaces are porous due to the leaching out of alkali ions such as lithium (electrode glasses contain mostly silicon, lithium, and calcium oxides). This porosity has the effect of exposing many more silanol sites than

would be possible at a single plane. The subject of the pH sensitivity of a glass electrode is thus quite complex and contains many themes which will recur in the explanation of the pH sensitivity of EIS structures. These are the question of bulk vs. surface theories and the precise definition or nature of the surface.

The thinking behind the original discovery of the ISFET in 1970 was that an SiO_2 surface was pH sensitive in a fashion analogous to that of a glass electrode. This analogy continued to be the preferred explanation during the next decade, although other suggestions were made, such as changes in surface-state density at the Si/SiO_2 interface. The problem with the glass-electrode-analogy explanation was that glass electrodes have a sensitivity typically at least 95% of Nernstian (between 56 and 59 mV/pH), whereas SiO_2 -gate ISFETs have sensitivities typically varying from 30 to 40 mV/pH. Silicon nitride gate ISFETs were reported to have sensitivities closer to Nernstian. Clearly, a quantitative theory to explain these findings was needed.

Two types of quantitative theories were advanced in 1979, each of them based on previous work on oxide surfaces by colloid scientists. The first, initiated by Lauks [24], assumed that the outer 30 to 100 Å of the insulator are porous and permeable to both anions and cations from solution (I will call this a porous-layer theory). This was based on work in colloid science by Lyklema [25]. The pH sensitivity of a surface is predicted to have a minimum at pH 7. The second theory, first proposed by Siu and Cobbold in 1979 [26], was based on the reactions of reactive sites at the insulator surface with ions in solution. In colloid science, this was first proposed by Yates et al. [27] and is often referred to as the site-binding theory. In surface-site theories, there is a clear connection between the parameters measured on ISFETs and on colloids. In 1983, it was demonstrated [28,29] that for both silicon dioxide and aluminum dioxide there was a good agreement between the parameters measured on ISFETs and those determined with suspensions of colloidal particles. This proved to be a strong argument in favor of surface-site theories, and these have since been adopted by many authors [30–33].

The arguments in favor of surface-site theories for explaining the pH-sensitivities of insulator surfaces can be listed as follows:

1. The presence of surface sites such as silanol on SiO_2 has been proved by a variety of methods, such as infrared spectroscopy.
2. The theory provides good agreement between colloid science and EIS measurements.
3. Porous-layer theories do not provide any testable connections with colloid science.
4. Porous-layer theories predict a sensitivity minimum at pH 7, which is not observed.
5. The response of an insulator to pH steps has been measured to be very rapid [5,34], in the millisecond range. Given the diffusion coefficients of ions in insulators [35], porous-layer theories cannot explain such rapid response.
6. The surface-site theory satisfactorily explains measurements of the capacitance at the insulator/electrolyte interface [10].
7. Capacitance measurements of oxides 100 Å thick show no signs of a porous layer, which should be very evident if it existed.
8. The surface-site theory can be extended to explain the behavior of surfaces with several types of site, such as silicon nitride [32] or chemically modified surfaces [30].
9. The surface-site theory satisfactorily explains the temperature dependence of the surface potential [36].

One striking aspect of this list is that surface-site theories are able to explain many aspects of the insulator/electrolyte interface: dependence on the nature of the surface, capacitance, temperature dependence, time dependence. The very rapid time response leaves little doubt that most of the time response must come from the surface and not from the bulk.

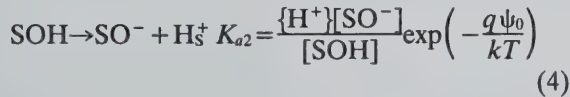
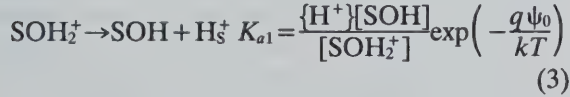
However, second-order phenomena occur at a slower time scale and are not explained by surface-site response theories. These phenomena are drift and hysteresis (and in many measurements these can be difficult to separate). Drift is a slow and continuous change of the flat-band voltage, independent of any pH changes. Hysteresis is a memory effect seen when the pH is cycled. It is shown below that hysteresis is caused by slow response to a pH step. The explanation of this slow response does not follow immediately from the surface-site theory. It has been suggested [35] that it is caused by the presence of active sites in the region of the insulator close to, but below the surface. The presence of OH groups in the bulk of SiO_2 is known using several different methods, and SiO_2 surfaces do show large amounts of drift and hysteresis.

The surface-site theories have been developed in detail in several papers, and I will not repeat this here. I will give more attention to recent work that examines the second-order slow-response effects. This slow response constitutes the greatest obstacle to the use of accurate pH sensors. This aspect of the pH response of insulators will receive more attention in the future.

2.2.2 Surface-Site Theory of pH Sensitivity

This section will give a summary of the surface-site theory of the pH sensitivity of insulators; for more details, references [28,29,37] should be consulted.

Oxides are assumed to have only one type of surface site, to be written as S-OH where S represents the element whose oxide is considered. This site behaves amphoterically: it can both gain and lose an H^+ ion. These surface acid/base reactions, with their associated equilibrium constants, can be written as:



where $\{H^+\}$ is the H^+ activity in the bulk solution, H_s^+ represents a hydrogen ion at the oxide/electrolyte interface, and quantities in square brackets are numbers of surface sites per unit area. The potential is the potential difference seen by a hydrogen ion as it moves from the solution bulk to the location at the interface where it charges an OH group.

The properties of an oxide surface are largely determined by the value of the two surface reaction equilibrium constants, K_{a1} and K_{a2} . The pH at which the surface is uncharged is given by:

$$pH_{pzc} = \frac{(pK_{a1} + pK_{a2})}{2} \quad (5)$$

and is known as the pH at the point of zero charge. Another important parameter is called ΔpK and is defined as:

$$\Delta pK = pK_{a2} - pK_{a1}. \quad (6)$$

This parameter is a measure of the tendency of the surface to become charged. When it is high, the surface is very unreactive and tends to have a poor pH response. This is the case for SiO_2 , where

$\Delta pK \approx 7$. Surfaces that become more easily charged and have a better pH response tend to have a lower ΔpK , such as Al_2O_3 , where $\Delta pK \approx 4$.

It can be shown [28,37] that around the point of zero charge, the pH response can be written as:

$$2.303(pH_{pzc} - pH) = \frac{q\psi_0}{kT} + \sinh^{-1}\left\{\frac{q\psi_0}{kT\beta}\right\} \quad (7)$$

where b is a dimensionless parameter that describes the pH sensitivity of a surface. Its value depends on ΔpK , the density of sites on the surface, and the double-layer capacitance, as follows:

$$\beta = \frac{2q^2 N_s 10^{-(\Delta pK/2)}}{kTC_{DL}} \quad (8)$$

where N_s is the number of sites on the surface per unit area, and C_{DL} is the capacitance of the double layer per unit area. When b is large, the second term on the right-hand side of (5) becomes negligible, and the equation reduces to the Nernst equation. Around the point of zero charge, the slope of the potential/pH curve is $b/(b+1)$ times the Nernstian value (this is one method of experimentally determining the value of b). Figure 40 shows the relationship predicted by eq (8) at various values of b .

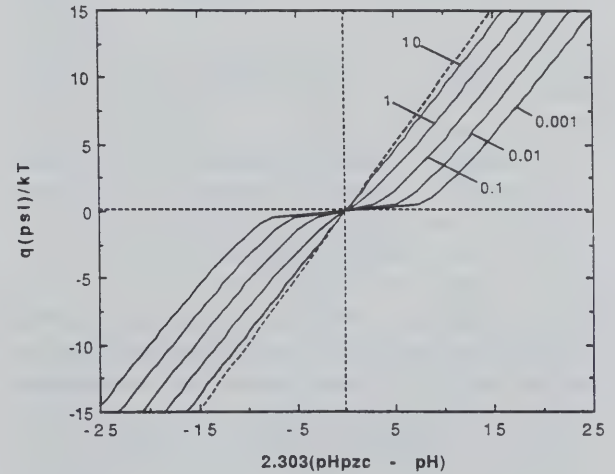


Figure 40. Surface potential/pH relationship for an oxide surface for various values of the sensitivity parameter b .

The advantage of the use of a parameter such as b is that it combines the effects of the various influences on the pH sensitivity into one number. The equation describing the pH response depends on only two parameters, which makes it easier for theory to be fitted to experiments. This formulation

also shows that it is impossible to determine the parameters ΔpK , N_s , or C_{DL} individually from y_0/pH measurements alone.

The results above were derived for the simplest possible case: an oxide surface with a single type of amphoteric site. The surface-site theory can be extended to deal with more complex situations. In particular, another set of reactions to consider is the adsorption of the counterion ions in solution on oppositely charged sites. This counter-ion adsorption, or complexation, can be shown to have a very small influence only on the potential/pH curves (although charge/pH characteristics of colloids are greatly affected) [29,37].

Another more complex case of considerable practical importance concerns surfaces with two different types of site. The most commonly used surface of this type is silicon nitride, whose surface contains silanol (Si-OH) and amine (Si-NH₂) sites. Another example is borosilicate glass, with B-OH and Si-OH sites. The surface-site theory can be extended to cover these cases [32]; in general the result is that the pH sensitivity is increased when the pH_{pzc} values of the individual types of site are very different. It is possible to define an equivalent b for the surface in terms of the individual b values of the two types of site:

$$\beta^2 = \beta_1^2 + \beta_2^2 + (10^{\Delta pH_{pzc}} + 10^{-\Delta pH_{pzc}})\beta_1\beta_2 \quad (9)$$

where ΔpH_{pzc} is the separation between the points of zero charge of the two types of site on the surface. For silicon nitride, that separation is very large, because the amine sites are neutral at about $pH=10$ and the silanol sites at $pH=2$. Therefore, it is predicted that for Si₃N₄ the equivalent b is very large, and the pH response therefore Nernstian. This is indeed mostly what is observed.

2.2.3 Hysteresis and Slow Response The previous section has considered the surface-site theory of the pH response of insulator surfaces. This theory is successful in many respects, as shown above, but does not by itself explain all aspects of the behavior of pH-sensitive EIS structures. Because the surface-site models have been in use for some time now, and their application to the modeling of chemical sensors has been discussed in papers by many authors, I will deal at greater length with phenomena not readily explained by surface-site models.

The main category of unexplained effects involves the time response to pH changes. When the pH of the solution in contact with an EIS sensor is changed abruptly, almost all the response to that

step occurs very rapidly (faster than 10 ms), as has been shown by Flow Injection Analysis experiments [5]. But a small part of the response is much slower, in the range of minutes to even hours. This slow response will cause hysteresis effects when the pH is cycled, as will be covered in more detail in this section. The other important nonideal time-response phenomenon is the long-term drift, which will be the subject of the next section.

A mathematical model that links slow response to a pH step and hysteresis has been presented in [38]. The purpose of this model is to describe systems whose response contains an element of delay. The delay is described by assuming that the step response of the system contains a first-order exponential term that is of the type $(1 - \exp(-t/\tau))$. Let's call e the portion of the response to a step function that is delayed with a time constant. This implies that a fraction $1-e$ of the response is immediate. The following discussion will make use of normalized units for both input and output steps; assuming that the input to the system is a pH step normalized to unity, then the normalized output function is assumed to be

$$\begin{aligned} O(t) &= (1 - e) + e(1 - \exp(-t/\tau)) \\ &= 1 - e \exp(-t/\tau). \end{aligned} \quad (10)$$

The normalization of the output is the obvious one—namely, such that the output after an infinitely long time to an input of unity is also unity.

It is well known that problems involving the response of systems such as the one described by the equation above, to various inputs, are best handled by operational calculus. Therefore, we need the Laplace transform of the output function above, which is

$$O(s) = \frac{1}{s} - e \frac{1}{s + \tau^{-1}}. \quad (11)$$

The transfer function of the system is defined by the ratio of output to input functions; because the unity step function is $1/s$ in the Laplace domain, the transfer function is

$$T(s) = 1 - e \frac{s}{s + \tau^{-1}}. \quad (12)$$

As expected, this transfer function reduces to unity in the ideal case that e is zero.

The problem is now of using this representation of a system with slow response to calculate how

much hysteresis it will cause. Hysteresis is the result of applying a periodic input pattern such as a sine wave or triangle wave. If the output is then plotted as a function of the input, a characteristic cigar-shaped hysteresis curve results. The best system to analyze is the triangle-wave input, because it is a good representation of an experimental protocol where the pH is swept first in one direction, then back in the other. The input to the system will thus be assumed to be a periodic triangular waveform, with amplitude going from 0 to 1, and with period $2t_s$ (t_s is the time needed for a sweep from one pH extreme to another). The Laplace transform of the input waveform is given by [39]:

$$I(s) = \frac{1}{t_s s^2} \tanh\left(\frac{t_s s}{2}\right). \quad (13)$$

And the transform of the output is thus:

$$\begin{aligned} O(s) &= I(s) \cdot T(s) \\ &= \left[1 - \epsilon \frac{s}{s + \tau^{-1}}\right] \left[\frac{1}{t_s s^2} \tanh\left(\frac{t_s s}{2}\right)\right] \\ &= I(s) - H(s) \end{aligned} \quad (14)$$

where $H(s)$ describes the deviation of the output waveform from the input triangular shape. This function thus describes the hysteresis behavior of the system, and is given by:

$$H(s) = \frac{\epsilon}{t_s} \frac{1}{(s + \tau^{-1})} \tanh\left(\frac{t_s s}{2}\right). \quad (15)$$

The problem now consists of finding the inverse Laplace transform of this function, which can be accomplished using the convolution theorem, with the result:

$$\begin{aligned} H(t) &= \epsilon \frac{\tau}{t_s} \exp(-t/\tau) \left(\frac{\exp(t_s/\tau) - 1}{\exp(t_s/\tau) + 1} + (-1)^n \epsilon \frac{\tau}{t_s} \right. \\ &\quad \left. \left\{ 1 - \exp(-(t - nt_s)/\tau) \left(\frac{2 \exp(t_s/\tau)}{\exp(t_s/\tau) + 1} \right) \right\} \right) \end{aligned} \quad (16)$$

where $n = [t/t_s]$, the number of sweeps of the input prior to the actual one. The first term on the right-hand side depends on t only through the factor $\exp(-t/\tau)$. This term, therefore, represents an initial transient in response to the turning on of the triangular waveform, which dies away with a time constant τ . It ensures that at $t = 0$, the output starts at 0, since $H(0) = 0$. The second term depends only on

$t - nt_s$, and represents the steady-state part of the response to a triangular waveform. It is a transient that depends on the time since the last change of slope of the input. As could be expected, that transient is the same for both n even or n odd, except for a change of sign.

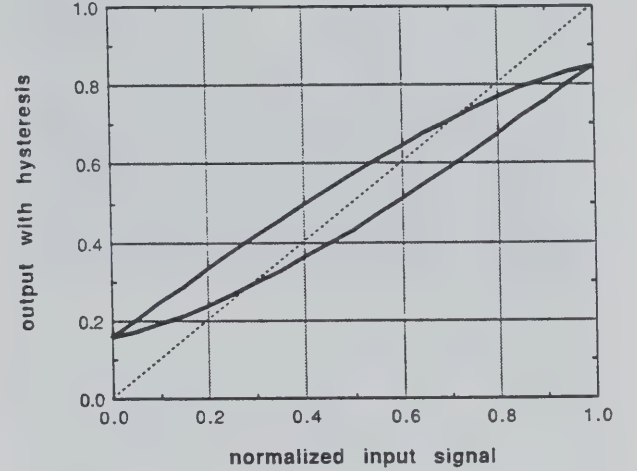


Figure 41. Theoretical hysteresis curve for the case $\epsilon = 0.4$ and $t/t_s = 0.5$. The value of ϵ is chosen unrealistically large to show the effect clearly.

Let's call the second term in eq (16) $H'(t)$. It is convenient to define a reduced hysteresis function $h(x, u)$, and to write:

$$H'(t) = (-1)^n \epsilon h\left(\frac{t}{t_s} - n, \frac{\tau}{t_s}\right) \quad (17)$$

where $h(x, u)$ is defined as:

$$h(x, u) = u \left\{ 1 - 2 \frac{\exp(-x/u)}{1 + \exp(-1/u)} \right\}. \quad (18)$$

Because the steady-state portion of the response depends only on the parity of n , we can take the two particular cases $n = 0$ and $n = 1$ to describe one complete hysteresis loop. When $n = 0$, it can be seen that the output/input relationship is explicitly given by:

$$O^+(I) = I - \epsilon h\left(I, \frac{\tau}{t_s}\right) \quad (19)$$

where I is the input as a function of time, and O^+ the resulting output. The equivalent equation in the second half of the loop, when $n = 1$, is:

$$O^-(I) = I + \epsilon h\left(1 - I, \frac{\tau}{t_s}\right). \quad (20)$$

Figure 41 shows an example of hysteresis when $e=0.4$ and $t/t_s=0.5$. It is apparent that the introduction of the first-order time constant has two effects. First, the average slope of the output is reduced. If we take the average slope to be that of the line connecting the two extremes of the loop, it is apparent that the slope is given by:

$$S = 1 - \epsilon \frac{2\tau}{t_s} \tanh\left(\frac{t_s}{2\tau}\right). \quad (21)$$

Figure 42 shows this reduction in slope as a function of the ratio t/t_s . When this ratio is low, the first-order response is fast enough to participate in the output, and the normalized slope is 1; when t/t_s is high, the first-order response is too slow to affect the output, and the normalized slope is $1-e$.

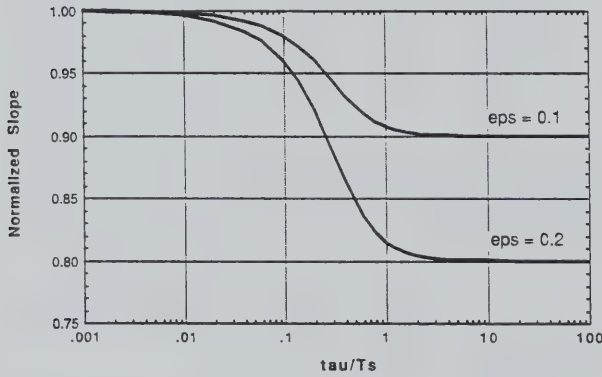


Figure 42. Reduction in the sensitivity of a sensor as a function of the ratio t/t_s .

The other important characteristic of the hysteresis loop is its maximum width, which by symmetry occurs when I is equal to 0.5. From eqs (12) and (13) it follows that the width of the hysteresis curve is:

$$W = 2\epsilon h \left(0.5, \frac{\tau}{t_s}\right). \quad (22)$$

Figure 43 shows how the width W depends on the ratio t/t_s . This figure is reminiscent of a resonance phenomenon: the hysteresis is maximum when the time constant of the slow response and the sweep time t_s are of the same order of magnitude. More precisely, the maximum occurs when $t=0.332 t_s$. When the time constant of the slow response process is either much higher or much lower than the sweep time t_s , the hysteresis tends toward zero.

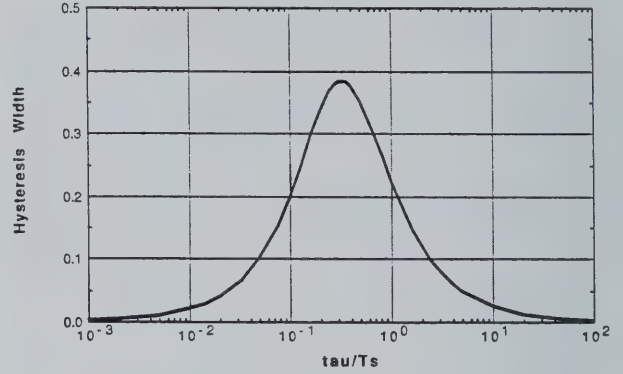


Figure 43. Dependence of the hysteresis width W on the ratio t/t_s .

The previous paragraphs have established the link between a first-order slow time constant in the system response and the magnitude of the hysteresis effect. A more general case is the presence of many first-order time constants. The transfer function of the system is then:

$$T(s) = 1 - \sum_i \epsilon_i \frac{s}{s + \tau_i^{-1}}. \quad (23)$$

Because the system is linear, though, each of these time constants will produce a term in the output given by eq (14). The analysis above applies to each term, and the results can be summed. In particular, the total hysteresis will be given by a sum of terms as given by eq (22):

$$W = \sum_i 2\epsilon_i h \left(0.5, \frac{\tau_i}{t_s}\right). \quad (24)$$

Figures 44 and 45 show the hysteresis behavior of two surfaces commonly used as pH sensors: aluminum oxide and silicon nitride. These results show that the magnitude of the hysteresis is similar for both surfaces. The details of the behavior are different: in Al_2O_3 , the hysteresis tends to be worse for alkaline pH excursions, whereas for Si_3N_4 the hysteresis loop tends to be broader on the acid side. But in both cases, the basic limitation of the obtainable accuracy is roughly the same: 7 mV, or 1.5% of the total excursion for Si_3N_4 , and 4.9 mV or 1.2% for Al_2O_3 . This limitation means that pH-sensitive EIS structures are not yet as accurate as glass electrodes, until an insulator is found with better hysteresis properties. However, there are applications where this lower accuracy is outweighed by other advantages of EIS structures. Some of these will be discussed in sections below.

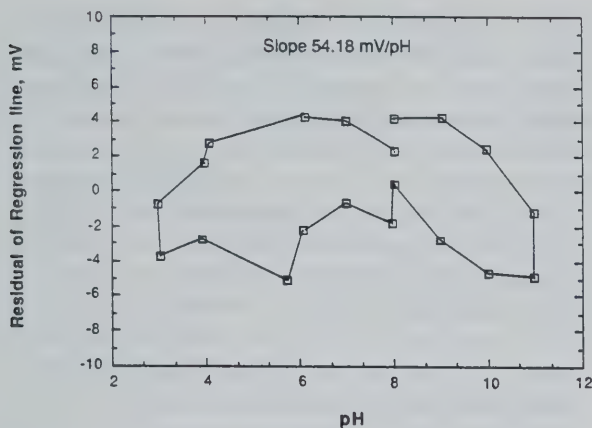


Figure 44. Experimental data for the hysteresis behavior of an Al₂O₃—gate ISFET.

In the case of the nitride surfaces we have made measurements both of the slow response (fig. 46) and of the hysteresis (fig. 44), on the same device. The slow response is modeled by three first-order exponential terms, as shown in eq (22), and the fit is given by:

$$O(t) = 120.9 \left(1 - 0.0089 \exp\left(-\frac{t}{450}\right) - 0.0212 \exp\left(-\frac{t}{2010}\right) - 0.0436 \exp\left(-\frac{t}{11890}\right) \right) \quad (25)$$

with the response in millivolts and time in seconds. This theoretical curve is also shown in figure 46. A more sensitive and useful way of representing the same data is to plot the derivative of the slow response—i.e., the drift rate—as a function of time in a log-log plot. This is shown in figure 47. Again, the curve derived from eq (25) represents the data very accurately down to drifts in the range of the background drift rate.

The model given above can be used to make a connection between these two phenomena. For each of the three time constants, a contribution to the normalized hysteresis width can be calculated according to eq (22). This leads to a total predicted hysteresis width of 5.2 mV, which is in reasonably good agreement with the 7.1 mV actually measured.

In the context of the surface-site model of explaining the response of insulator surfaces [28,29,37], the slow response can be explained by assuming that some sites react slowly to changes of pH [35]. This slow reaction could be due to the location of the site relative to the insulator surface,

for instance, if some roughness or microscopic porosity is present. Another possibility is that some sites could be located in the bulk insulator just beneath the surface [35]. For instance, silicon nitride contains a certain amount of hydrogen in its bulk, depending on the deposition method. This creates NH groups in the nitride that are similar to secondary amines. Groups of that type located close enough to the surface might be able to participate in a slow equilibrium with protons in solution, and acquire a positive charge. In that case, we would expect low hydrogen contents to be favorable for good pH responses. That has, in fact, already been observed with Al₂O₃ [40]. In the case of nitride deposition, ammonia is the source of the hydrogen incorporated in the film. Thus, LPCVD nitride, which is usually deposited with ammonia/dichlorosilane ratios in the range of 3 to 10, is expected to have a lower hydrogen content than atmospheric-pressure CVD nitrides, where the ammonia/dichlorosilane ratio is more typically 100. This correlates with the observation that LPCVD nitride is a better pH-sensing material than APCVD nitride [16]. Another example of that observation is that the LPCVD nitride described by Bousse et al. [16] has a long-term drift value about 10 times lower than Matsuo's APCVD nitride, described in reference [41].

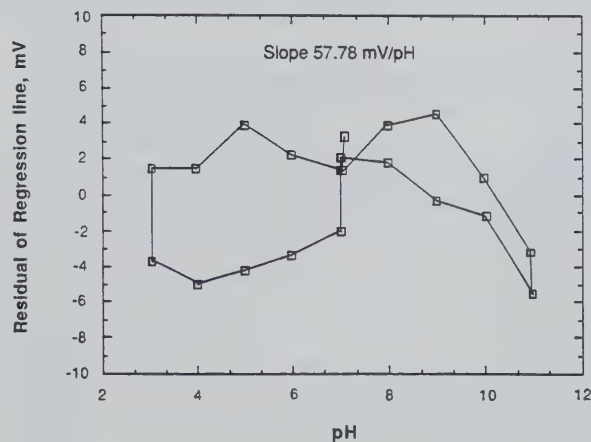


Figure 45. Experimental data for the hysteresis behavior of an Si₃N₄—gate LAPS device.

In summary, better insulators will be needed to improve the accuracy of pH sensing. A convenient test is to measure the slow response to a pH step. Better insulators can either be new materials, such as tantalum oxide, which has been reported to have good properties [42], or known insulators deposited in a better fashion. It is important to re-

member, for instance, that there is a wide variety of silicon nitrides, with varying properties. The plasma nitrides have high hydrogen contents and low densities and are unlikely to be suitable pH sensors. High-temperature LPCVD-deposited nitride, on the other hand, is one of the better pH-sensing insulators known. So improvement can be sought either in new materials or in optimizing the details of the deposition conditions of a known material.

2.2.4 Long-Term Drift of EIS pH Sensors It is important to distinguish true long-term drift from the effect of very long time constants in the pH response. The previous section shows that at least 12 h of stabilization are needed to accomplish this. After that, drift tends to be slow and decreases constantly. A typical empirical description of drift is:

$$V = V_0 + a \log\left(1 + \frac{t}{t_0}\right) \quad (26)$$

where V is the sensor output voltage, and t is the time. This equation describes a process that does not have an asymptote, and the output voltage is not bounded with time. However, the drift rate continually decreases, and goes as $1/t$ for time much greater than t_0 . This has been verified for Al_2O_3 and indicates that drift can become as low as needed provided enough time is allowed to pass.

Most data on long-term drift have been compiled by Matsuo and Esashi [41]; they show that the order from best to worst is $\text{Ta}_2\text{O}_5 > \text{Al}_2\text{O}_3 > \text{Si}_3\text{N}_4 > \text{SiO}_2$. The drift for silicon dioxide is particularly high and does not show the logarithmic behavior of the previous equation.

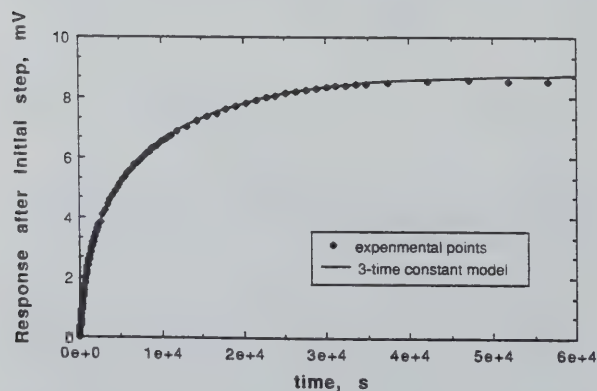


Figure 46. Experimental data for the slow response of a Si_3N_4 -gate LAPS device to a pH step from 7 to 9.

2.2.5 Overview of pH-Sensing Properties of Si_3N_4 , Al_2O_3 , and Ta_2O_5 Si_3N_4 , Al_2O_3 , and Ta_2O_5 are the insulators most often used in EIS pH sensors. All have good pH response properties and are candidates for real-world commercial applications. The response slope is about 50 mV/pH for Al_2O_3 , 55 mV/pH for Si_3N_4 , and 55 to 58 mV/pH for Ta_2O_5 . These are all good numbers, and on this basis alone little distinction can be made between the three materials. It should be noted that the silicon nitride is assumed to be deposited by LPCVD; it is known that this material is superior to nitrides obtained with atmospheric-pressure CVD.

The difference between these materials lies essentially in the hysteresis and drift properties. These have been studied for silicon nitride [38] and aluminum oxide [43], and the conclusion is that the amount of hysteresis and drift is very similar for Al_2O_3 and Si_3N_4 . Because nitride is much more commonly available than aluminum oxide, it would appear to have an edge. Tantalum oxide appears to have clearly less drift and hysteresis than Al_2O_3 and Si_3N_4 (perhaps by a factor of three). Its use appears clearly advantageous, and it is probably the best pH-sensing insulator known at present. There is one possible drawback to its use: the usual method of depositing it is the thermal oxidation of a thin film of metallic tantalum. This process causes fairly high pinhole densities. In the case of an FET, where the gate area can be very low ($10 \times 100 \mu\text{m}$ would be typical), high defect densities can be tolerated. But it might be an obstacle to the application of Ta_2O_5 in a LAPS device.

2.3 Use of Ion-Selective Membranes on Top of an EIS Structure

One line of research that has been explored extensively by Janata and his coworkers is the coating of the insulator of an ISFET with an ion-selective membrane. The result is an FET sensor sensitive to a given ion, and it is these devices that Janata has called CHEMFETs. I will not go into detail about this work, because Janata has written several extensive reviews about it [17,18]. In general, the same type of membranes used in ion-selective electrodes has been applied as coatings to the gate of an ISFET.

2.3.1 Examples of the Many Types of CHEMFETs Most CHEMFET membranes are polyvinyl chloride (PVC) with a plasticizer and an ionophore for cations. The usual ionophores are valinomycin for potassium, nonactin for ammonia, and various synthesized compounds for sodium, lithium, and

calcium. Professor Simon's research group at the Technical University in Zürich is the world leader in the synthesis of such compounds. Several books have been written about this type of ion-selective membrane [44], and they are commercially available through Fluka.

Other types of membrane made for CHEMFETs are solid, such as silver halides to detect halogen ions, or heterogeneous membranes: elastomers in which solid particles are mixed. Shiramizu et al. demonstrated sensors of this type using silver halide particles [45].

2.3.2 Problems: Membrane Adhesion, CO₂ Sensitivity Membrane-covered EIS structures, which are almost always ISFETs, suffer from a number of serious problems, which are serious obstacles to their practical application. Two important ones are:

1. Lack of adhesion of the membrane. PVC membranes do not adhere very well to oxide surfaces or to silicon nitride. The usual failure mode is that the aqueous solution penetrates between the PVC and the gate insulator and shorts out the PVC membrane. This often happens within days. Several groups are working on novel membranes with better adhesion properties in order to avoid this problem.
2. Tab sensitivity to CO₂ in the atmosphere. This occurs because the PVC is gas permeable, and nothing is at the PVC/insulator interface to buffer the pH there. Therefore, even small amounts of CO₂ alter the pH at that interface, which causes a drift in the sensor [49].

2.3.3 Suggestions for Solutions to these Problems The problem with the insulator/PVC interface is that there are no common charge carriers on either side, and no thermodynamic equilibrium can be established. This interface is therefore unstable and vulnerable to any factor that can affect the local charge state of the surface sites, which depends in turn on the activity of hydrogen ions at the surface. A stabler situation is to put an electrolyte between the PVC and the insulator. This electrolyte must be buffered in pH and must contain a large concentration of the ion the device is trying to detect. This intermediary electrolyte creates two new interfaces whose potential is well defined: the insulator/electrolyte interface (fixed by the pH of the electrolyte) and the membrane/electrolyte interface (fixed by the concentration of the ion for which the membrane contains an ionophore). This method has been used by van den Vlekkert [50] and leads to very good stabilities.

The interesting part about this type of structure is that the EIS part of the device is again used as a pH sensor, although the entire device is sensitive to another ion. Thus, it appears that the EIS device should *always* be thought of as a pH sensor, even in structures that as a whole are intended to sense other quantities.

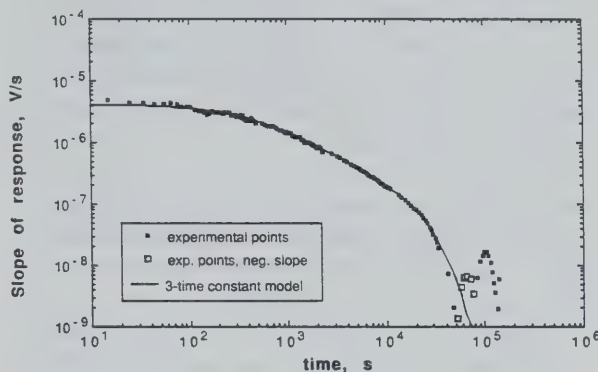


Figure 47. A different presentation of the same data as in 2.13: the time derivative of the pH response (i.e., drift rate) shown in a log-log plot.

2.4 Trends in Research on EIS

2.4.1 Competing with Ion-Selective Electrodes

As noted above, much of the early work on ISFETs and CHEMFETs produced devices which are in essence a type of ion-selective electrode. The membrane types and ionophores used all came from research work originally done on conventional ion-selective electrodes.

There have been several ISFET or CHEMFET devices which have been advanced to a fairly high degree of development. I will only cite a couple of examples that are particularly relevant in terms of biological applications. The first is the catheter tip pH sensor developed by Cordis Europa in the Netherlands [51]. That device was successfully tested in animals, and advanced to the stage of clinical testing. It was capable of sufficiently accurate pH measurement that breath-to-breath pH variations could be monitored. It was never marketed however; some possible reasons for that are problems with biocompatibility, and high production costs compared to the cost of a test on a conventional blood gas analyser. Another drawback is that clinicians are accustomed to basing their diagnosis on the value of all three "blood gases": pH, pCO₂, and pO₂. Providing only one of the three is viewed as insufficient for clinical purposes. Another example is the four-function multisensor intended for clinical use described by Sibbald et al.

[52]. This device was not intended to be *in vivo*, which reduces biocompatibility problems, but does provide four different measurements, namely for potassium, hydrogen, calcium, and sodium ions. It is a miniature flow-through cell containing an integrated circuit with four CHEMFETs. The intent is to sample blood from a patient, or run a continuous small blood flow through it.

Up to now, ISFET or CHEMFET devices have not shown a sufficiently clear superiority over conventional ion-selective electrodes to become successful in the marketplace. The reasons can be summarized as follows:

1. Since the membranes are typically the same, the chemical performance of a CHEMFET cannot be better than the ion-selective electrode.
2. The fabrication cost is higher, because the silicon chips are not produced in large quantities (extremely high volumes are needed to obtain economies of scale in silicon fabrication).
3. There are problems of reliability, carbon dioxide sensitivity, and membrane adhesion.
4. The cost of the devices is limited by that of the competing ion-selective electrodes, and is not high enough to justify the high research and development expense of making the CHEMFETs.

The situation is a little different for pH-sensing ISFETs; these compete with glass electrodes, which are cheap but have a few disadvantages such as high output impedance, slow response, and large size. There are some specialized applications where an ISFET has advantages over glass electrodes; an example is *in vivo* use. However, the issues of biocompatibility and causing of blood clotting have deterred actual clinical use.

The general conclusion is that attempts to make EIS-based chemical sensors compete with conventional ion-selective electrodes have not been successful. There are not enough advantages to justify a change. This means the potential value of working with silicon-based sensors has to be sought in something other than an alternative type of ion-selective electrode. I believe that a clear trend has started toward using EIS sensors as part of more complex systems in which the silicon has other useful functions. Examples of this will be discussed in the sections below. These applications are typically biological or biochemical and fall within the realm of what is usually called a biosensor.

2.4.2 Who is Working on These Devices The ISFET is a European invention that was very quickly taken up in Japan. These two areas are still

the ones where most work is currently being done. Attempts in the USA to commercialize CHEMFETs were not successful and led to disenchantment there. Today, little or no work is being done in the USA on FET-based sensors for ionic concentrations. In Japan, however, several major corporations (Hitachi, Toshiba, and NEC, for instance) have active research programs. Much work in Japan is done on enzymatic multisensors, and Japanese researchers appear to have little interest in sensors for simple ions such as potassium, sodium, and ammonium. I expect that in due course, the results of the Japanese work will find itself in commercial products. The same is less likely to happen in Europe, because most of the work there is done in academic settings, and large corporations have not shown much interest.

The driving force behind this research is of course the eventual application, and most attention has gone to biomedical or biological applications. Projects in the area of analytical chemistry or industrial process control have not gone very far. There seems to be a consensus that the biological area is the one where novel sensor devices are most likely to have an impact.

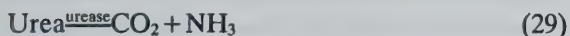
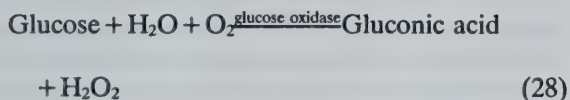
3. EIS Sensors Used in Combination With Enzymes

Much work has been done during the past decade on the combination of solid-state pH sensors and enzymes that catalyze biochemical reactions. The role of the enzyme is to provide a specific sensitivity to certain biochemical molecules which the sensors discussed previously are not able to detect. The result is an example of a biosensor, which is usually defined as a sensor specifically sensitive to a biological molecule. A review of the general subject of using enzymes in biosensor applications has been written by Guilbault [53].

An enzyme is a protein that acts as a catalyst. That is, it enables a reaction to occur at a significant rate. The substances whose reaction is catalyzed by an enzyme are substrates. I will often use as examples two enzymes that occur several times in the material to follow and are very commonly used in biosensors: glucose oxidase and urease. The substrate of glucose oxidase is glucose; for urease it is urea. The general reaction of an enzyme with its substrate can be written as:



where E denotes the enzyme, S the substrate and P the product. The reactions catalyzed by glucose oxidase and urease are:



Most enzymes have several substrates, and will act on some other substances than their usual biological target. For instance, urease also catalyzes the decomposition of formamide, although at a much lower rate than for urea. In the context of biosensors using enzymes and semiconductor sensors, there is a danger of confusion between two completely different uses of the word substrate. It has meaning for the enzymologist; in addition, the device physicist uses it for the bulk semiconductor in which a device is fabricated. In this section, I will always use the word in its enzymological meaning.

Many enzymes require the presence of other substances to function. In many cases, a metal ion needs to be present for the enzyme to be active. Other substances required in the enzyme reaction are cofactors or coenzymes. Typical examples of biological molecules that can act as cofactors are the adenosine phosphates (AMP/ADP/ATP) or nicotinamide adenine dinucleotide phosphate (NAD). Some cofactors act as co-catalysts because they are not used up in the enzyme-catalyzed reaction itself; others are also substrates and are used up stoichiometrically in the enzymatic reaction.

Enzymes are isolated and purified from a variety of sources, such as plants or bacteria. For example, the most common source of urease is a plant (jack bean) [54]; however, many bacteria also produce ureases [55]. Bacterial ureases have structures somewhat different from that of jack bean urease, but with many overall similarities [56]. Harvesting and purifying an enzyme from a bacterial culture can be difficult, but this process can be facilitated in some cases by genetic engineering. In the case of urease, it has been reported [55] that the gene for a given bacterial urease (from the organism *Providentia stuartii*) has been expressed in *E. Coli* bacteria; a culture of these modified organisms can then provide a more plentiful and convenient source than the organism from which the gene was taken. This particular urease is reported to be about three times faster than the standard jack bean urease; however, it is not available commercially or in larger quantities.

We will discuss two types of enzyme-based biosensors. First come the devices in which an immobilized enzyme is used to catalyze a reaction of the analyte, and an electrochemical sensor is used to detect one of the reaction products. These devices are enzyme electrodes, the best known examples of which are glucose electrodes with immobilized glucose oxidase. This approach requires that the analyte of interest is a substrate of an enzyme and that the reaction has easily detectable reaction products. Next, we will discuss systems in which an enzyme is used as a label attached to another molecule, typically an antibody, that binds to the substance of interest. This is a more complex system, but has the advantages of higher sensitivity and the ability to detect more substances obtained with antigen/antibody binding.

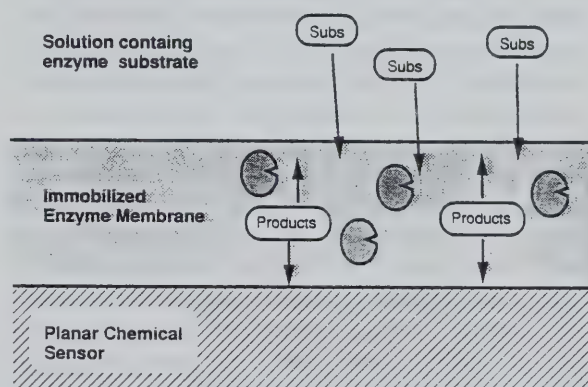


Figure 48. General concept of a biosensor with an immobilized enzyme membrane.

3.1 Sensors with Enzyme-Containing Membranes

3.1.1 Description of General Concept The enzyme-containing membrane is the key part of devices of this type. This layer must be relatively thin and have immobilized in it an enzyme that catalyses the decomposition of a substrate whose concentration one desires to measure. This layer must also allow diffusion of the substrate molecules. The enzyme then produces the reaction products, and an ion-selective sensing device placed immediately underneath the membrane detects one of these reaction products. This general concept is illustrated in figure 48.

The function of the enzyme layer is to act as a transducer between the biochemical world and the electrochemical one. It translates the concentration of a biological molecule into a concentration of an intermediate that can be detected with an electrochemical sensor of some kind.

If one of the reaction products is a dissolved gas (such as ammonia, in eq 29), an ammonia gas sensor could be used. If oxygen or hydrogen peroxide is involved, as is the case with glucose oxidase eq (28), amperometric sensors with noble metal electrodes are suitable. Reactions that change pH can be detected by potentiometric devices. Because the enzyme reactions rarely involve cations such as sodium or calcium, ion-selective membranes for such ions are not useful for enzyme electrodes. The most common potentiometric sensor applications in this context are for the measurement of pH and NH_4^+ ions.

Many enzymatic reactions can be detected by several different underlying electrochemical sensors. For instance, in the oxidation of glucose (reaction 28), the production of hydrogen peroxide or the consumption of oxygen can be detected amperometrically at noble metal electrodes (at different potentials), and the acidification induced by the generation of gluconic acid can be detected potentiometrically with a pH-sensitive EIS structure. The decomposition of urea can be followed by an ammonia gas electrode, a NH_4^+ ion-sensitive electrode, or a pH-sensitive electrode (since most of the ammonia will react with water to produce OH^- ions).

I will only discuss further the application for potentiometric sensors, because the application of amperometric sensors is treated in another chapter of this series. In particular, I will make no attempt here to list all of the vast literature concerning glucose sensors. However, issues related to the immobilization of enzymes or to the underlying electrochemical sensor are to a large extent common to both potentiometric and amperometric electrodes. Thus, the first step is to present an overview of enzyme immobilization methods.

3.1.2 Methods for Immobilizing Enzymes To immobilize an enzyme on the surface of an electrode of any kind, at least two problems must be solved. The first is how to attach the enzyme to the electrode surface in a fashion that is robust (i.e. the enzyme does not leach away) and does not compromise the activity of the enzyme. These two requirements are clearly contradictory; because enzyme activity requires that the tertiary structure of the protein—its three-dimensional shape, be retained, it is difficult to covalently bind such a molecule to a surface without losing activity. The second problem to be solved is one of manufacturing technique; how to apply the enzyme-containing membrane to a selected area of a microstructure, preferably in a

fashion compatible with mass fabrication. I will discuss these two issues consecutively in this section.

There exists a considerable amount of literature concerning the immobilization of enzymes on structures larger than the micro-structures treated here. Typical for these “larger” applications are electrodes of a size comparable to glass electrodes, and bioreactors in which enzymes are immobilized on solid carriers such as glass beads. A comprehensive review of these applications of immobilized enzymes has been written by Guilbault [53]. He also describes a variety of immobilization methods. Most of the methods used for microstructures are identical to or inspired by techniques reported by Guilbault for larger structures.

Many researchers have been searching for improved enzyme immobilization techniques recently. As a result, many new methods are being reported in the literature. The main methods reported in the literature for immobilizing enzymes on microstructures are:

1. Physical entrapment in a hydrophilic polymer such as a polyacrylamide gel, as reported by van der Schoot et al. [57] and Aizawa et al. [58]. This method is among the gentlest for the enzyme and is extensively described by Guilbault [53]. The disadvantages are that there is some loss of the enzyme because it is not held in place with chemical bonds and that these membranes are inevitably rather thick (200 μm in the paper by van der Schoot et al. [57]).
2. Physical entrapment in a hydrophilic gel can be held in place by a cavity structure. The micro-cavity with small openings is intended to limit loss of enzyme and make the structure more robust to handle. This has been reported by Schneider et al. with agarose gels [59], using cavity structures of a type reported on earlier by Prohaska [60]. The cavities were not entirely successful in retaining the enzyme, and further cross-linking with glutaraldehyde (see next item) proved to be helpful. The use of cavity structures in the fabrication of microsensors has been reported initially for ion-sensing electrodes [61–63] but may be very useful for making enzyme sensors more reliable.
3. Covalent linking of an enzyme to a hydrophilic gel such as a polyacrylamide gel. This method is described by Caras et al. [64,65]. The enzyme is derivatized with N-succinimidyl methacrylate, mixed with an acrylamide/bis(acrylamide) solution that is then polymerized in the usual fash-

- ion. The result is a gel with the enzyme tightly bound to it, which should reduce the loss of enzyme considerably.
4. Immobilization in a bovine serum albumin (BSA) membrane crosslinked with glutaraldehyde (GA), a bifunctional reagent. This method has been used since the 1960s for immobilizing enzymes [53], but was first used for a microsensor by Yao [66]. Since then it has become one of the most widely used types of immobilized enzyme membrane [67–75]. The reasons for the popularity of this method are that it is relatively simple, it uses easily available chemicals, and it yields membranes which work reasonably well. The limitations of the method that researchers are striving to overcome, are slow response due to a relatively thick membrane, lack of reproducibility, and sometimes insufficient stability of the enzyme.
 5. Enzymes attached to an electrode surface by covalent linking, as reported recently by several authors [76–78] for amperometric sensors. Electrodes with covalently linked enzymes show faster response times; it is not yet clear if covalent linking can improve the sensor lifetime as well.
 6. Enzymes incorporated in an electrochemically grown polypyrrole film. This is a layer of a conducting polymer that can be grown in situ on an electrode. Enzymes are incorporated in such a film if they are present in the solution in which the polymerization takes place. This has been reported first by Foulds and Lowe [79] and Umana and Waller [80], and several authors have contributed publications since then [81–85].
 7. Langmuir-Blodgett (LB) films of an enzyme-lipid complex. This promising technique was recently described by Okahata et al. [86]. To make use of the LB technique, an amphiphilic substance is required that will spread out on an aqueous solution, and an enzyme cannot be used directly due to denaturation at the liquid-air interface. However, Okahata et al. [86] have shown how to make an enzyme-lipid complex that can then be coated onto a platinum electrode by the LB method. The LB film is only about 5 nm thick. The result is an amperometric glucose sensor with a rapid response, but most important, with a good lifetime (3 months). Significantly, membranes formed by casting of the same enzyme-lipid complex had a poor lifetime. This suggests that control of the microstructure of these very thin membranes is critical. An-

other LB film of glucose oxidase was made by Harrison et al. [87], by pretreating the glucose oxidase with glutaraldehyde, which makes it a more rigid structure without denaturing it. Then it can be spread out in a Langmuir through in the usual fashion, without denaturing it. The film can then be coated onto a solid surface as one or more monolayers. This yields a film that is very dense in the enzyme.

8. Immobilization in or behind a Nafion membrane. Nafion is the trade name of sulfonated fluorocarbons. It forms a film that is porous but because of the high negative charge on the sulfonate groups, cations cannot penetrate easily. Several groups have made amperometric sensors by dissolving some glucose oxidase into a Nafion film, or by spreading a Nafion film on top of a film made by one of the previous methods [88–90]. This method could have strong advantages in whole blood, because the Nafion is effective in keeping away the many possible interfering substances. Problems with lifetime and reproducibility remain, however.

The second issue is the manufacturing of microsensors with enzyme membranes. The problem is that many techniques for making these membranes involve operations done by hand on individual, packaged sensors. This loses all the advantages of the fabrication of many devices in parallel on one wafer (usually a silicon wafer). Cheap, mass-produced, possibly disposable (to avoid lifetime issues) enzyme sensors are only possible if the full economies of scale typical of the silicon integrated-circuit industry are achieved. Therefore, the key is to find methods for wafer-scale application and patterning of membranes. Many authors up to now have not addressed this issue at all, but the recognition that this is a crucial issue is growing, and a number of solutions have been proposed:

1. Incorporate the enzyme in a photosensitive membrane, which can be patterned in the same way as a photoresist. This method has the disadvantage of wasting almost all the enzyme used to cover a whole wafer, because the active areas typically cover only a small fraction of the area. Because many enzymes are expensive or not available in large quantities, this is an important issue.
2. Use a photoresist membrane to pattern the enzyme membrane with lift-off. This method has the advantage of not requiring a photosensitizing agent in the enzyme membrane, but also wastes most of the enzyme [72–75].

3. Use an ink-jet nozzle to deposit beads of enzyme-containing membrane solution on the active areas. This technique, reported by Kuriyama et al. [91], was specifically designed to avoid wasting enzyme. The resulting membranes have a very corrugated appearance, because they are a collection of beads.
4. In a similar method described by Anna et al. [92], a capillary tube, a pressure source, and an X-Y table are used to deposit round beads at selected locations.

It can be anticipated that research in wafer-scale manufacturing of enzyme membranes will increase, because a successful method is a prerequisite to commercial success. Many enzyme microsenors are for measuring entities such as glucose where alternative test methods already exist, and therefore the cost per test must be fairly low. Because of the intrinsically limited stability of most enzymes, the sensor's lifetime is unlikely to be long enough for many repeated uses. The only way to meet the requirements is a disposable microsensor, which is cheap to produce in large quantities. The integrated-circuits industry shows that this can indeed be done for very powerful electronic components; but it is dangerous to rely on this analogy too much. The IC industry took several decades to reach its present state and the volumes required for reaching low unit prices for components are very high. Thus, the barriers to commercial success in enzyme microsenors are high but not insuperable.

3.1.3 Application to EIS-Based Sensors All the published work describing sensors with an enzyme film over an EIS structure involve ISFETs. This is easy to understand, since field effect transistor devices are easy to make very small, so that small probes can be designed. In addition, it is common to use two devices side by side, one containing the enzyme, and one without the enzyme, but otherwise identical. The potential small size of ISFETs makes this easier to do in a small probe.

Devices of this type have been called ENFETs, and the first one was described by Danielson et al. [93]. Since then, Caras and Janata have been very active in this field, initially by describing a penicillin-sensitive device [94]. Subsequently, they presented a theory of the operation of these sensors [95], and presented supporting data for sensors for glucose [64] and penicillin [65]. The immobilization technique used was covalent binding to a polyacrylamide gel matrix (see item 3 of sec. 3.1.2).

Another early example of an ENFET was described by Miyahara et al. in 1982 [96]. The enzyme used was trypsin, immobilized by glutaraldehyde cross-linking to a cellulose membrane. This device responded to the presence of its substrate, but very slowly: the response time constant was of the order of two hours. Further work by this research group was described by Karube [97]: devices sensitive to urea (using urease) and to ATP (using $H^+ - ATPase$). The enzyme immobilization methods were similar, but the response times improved considerably, to about 2 to 5 minutes.

It is worthwhile to look into the Caras et al. theory [95] a little more deeply. The problem can be described in terms of the enzyme kinetics, the diffusion of the substrate into the membrane, and the diffusion of the reaction products out of the membrane. This leads to a set of coupled partial differential equations that have to be solved numerically. The solutions are different in the region of diffusion control (thick membranes), and reaction control (thin membranes). The model also includes the effect of the buffer capacity due to a buffering species assumed to be in the membrane. The main conclusions of the model are:

1. It is necessary to operate in the diffusion controlled mode, with thick membranes. The dynamic range is much smaller with thin membranes, since most substrate concentrations are sufficient to vary the surface pH enough to deactivate the enzyme. Note that this conclusion is only valid for potentiometric sensors; amperometric sensors consume one of the reaction products at the surface, and its concentration therefore cannot build up. This means that if we want to use an immobilization technique such as 5 to 7 above which leads to very thin membranes, we need to place another membrane on top solely for ensuring diffusion limitation.
2. The buffer capacity in the membrane plays an important role. The lower the buffer capacity, the lower the detection limit.
3. For typical diffusion coefficients, and a 1-mM buffer concentration, the detection limit is about 100 μM of substrate.
4. The response time of the sensor is roughly given by the sum of the diffusion time constants (L^2/D) for the diffusion of the substrate and the product through the membrane. The order of magnitude is around 10 seconds.

3.1.4 Limitations of This Concept Devices using an enzyme membrane on top of an EIS struc-

ture have certain intrinsic limitations to their sensitivity. The reactions used all generate products that change the pH, and the amount of pH change induced depends directly on the buffer capacity seen by the reaction products. To increase sensitivity, a low buffer capacity is needed. This means low concentration of pH buffers, but especially a low effective volume in which the reaction products are confined. One way to achieve this is to enclose the enzyme membrane in such a fashion that the reagents cannot escape. Unfortunately, that cannot be done because it would also stop the diffusion into the membrane of the substrate one wishes to detect. In other words, there is a trade-off between sensitivity and response time. This is a fundamental contradiction that can only be overcome by using means other than diffusion to introduce the substrate into the enzyme membrane. This means a system more complex than a device that can be dipped in a solution and then read.

The conclusion is that milli or micromolar sensitivity is the best that can be achieved with sensors of this type; higher sensitivity will require more complex systems.

3.2 Sensors That Use Enzymes as Detection Labels

3.2.1 Enzyme Activity in Low Volumes The previous section showed that the sensitivity of sensors that rely on membranes containing immobilized enzymes depends on the effective volume in which the products of the enzyme-catalyzed reaction are confined. This volume has to be relatively large, because these sensors are intended to be placed in a solution of the analyte of interest, and the reaction products can diffuse out of the membrane. In effect, the situation is diffusion from a point source into an infinite half sphere, where part of the diffusion is slowed down by a membrane. Clearly, a method for improving the detection limits would be to ensure that the enzyme-catalyzed reaction occurs in a very small volume. A low number of enzyme molecules will then be able to produce sufficient product molecules that their concentration is detectable, because concentration is inversely proportional to volume. Thus, we arrive at the concept of confining the reaction in a microvolume chamber.

There is of course a price to pay in obtaining a higher sensitivity by this method. The sensor is no longer a device that can be immersed in any solution of interest to obtain a direct reading. The sample must somehow be introduced in this

microvolume chamber. Therefore, the sensor will become part of a more complex measurement system. Also, in most cases a type of concentration step will be needed, because the microvolumes will typically be below one microliter, and usual sample sizes will be more in the milliliter range.

Another aspect to be considered is the generality of the measurement method. Basically, the sensors described above are limited to measuring the substrates (in this context, substrate means a substance whose reaction is catalyzed by the enzyme, such as glucose for glucose oxidase) of the immobilized enzymes. This is a rather limited category of analytes. A more general methodology is to use the enzyme as a label, by attaching it to another substance that binds to an analyte of interest. The most common example of such a method is the enzyme-linked immunoassay. This uses antigen-antibody binding reactions, that are very general because antibodies can be made to virtually anything. The key component in an enzyme-linked immunoassay is an antibody that is chemically linked to an enzyme in such a fashion that the activity of each molecule is retained. This enzyme can be chosen to be the most appropriate label for subsequent detection. Thus, the microvolume chamber will be used to detect these label enzymes, in order to quantitate the amount of antigen bound to antibodies linked to the enzyme.

In the sections below, we will consider one by one the issues involved in designing a sensor system using enzymes immobilized in a microvolume chamber: first, the analysis of enzymes (or other entities producing measurable chemical change) in small volumes, then the concentration of enzymes in such small volumes, and finally an overall description of a "sandwich" immunoassay system using these concepts.

3.2.2 General Analysis of Microvolume-Reaction Chambers Although this section is concerned with biosensors using enzymes, I will present a general analysis of the chemical change induced in a microvolume chamber by the generation of certain compounds. The analysis will use the specific case of the generation of H^+ or OH^- ions, that cause a change in pH. It is not difficult to expand the argument to cover other species. In general, we will assume that something in the microvolume chamber generates H^+ or OH^- ions at the rate R :

$$\frac{dn}{dt} = R \text{ (mol/s)} \quad (30)$$

where R is in the units of moles per second and is considered positive for the generation of OH⁻ ions and negative for the generation of H⁺ ions. Thus, a positive R will increase the pH, and a negative R will decrease the pH. We will assume in general that R does not depend on time, although it may depend on pH.

In the case that an enzyme is present to catalyze the reaction that generates the pH change, R is given by:

$$R = AE \quad (31)$$

where E is the mass of enzyme present, and A is its specific activity, in units of moles/gs. Note that the specific activity of an enzyme is different from the turnover rate; the specific activity is moles of product per mass of enzyme per second, and the turnover number is moles of product per moles of enzyme per second. Therefore, the turnover number has units of s⁻¹, and is equal to A multiplied by the molecular weight of the enzyme. We are assuming that the solution contains a large supply of all required substrates, so that the reaction rate is not limited by them. In terms of Michaelis-Menten kinetics, this means that the substrate concentration is much higher than the K_M of the enzyme, so that the reaction rate is equal to V_M , and does not depend on substrate concentration.

What we need now is the link between the number of moles of H⁺ or OH⁻ ions added to a volume, and the pH change caused by that addition. In chemistry, this is described by a quantity called the pH buffer capacity, and is defined by the following equation [98]:

$$\frac{dn}{dpH} = R \left(\frac{dpH}{dt} \right)^{-1} = V \beta_V \quad (32)$$

where dn is the number of moles of OH⁻ or H⁺ ions added to a solution, dpH is the resulting pH change, V is the volume of the reaction chamber, and β_V is the buffer capacity of the solution. This equation defines the buffer capacity to be a positive number, by virtue of the sign convention given above. When very small volumes are considered, it is necessary to consider the buffer capacity of the surfaces present, and to generalize eq (32) to:

$$R \left(\frac{dpH}{dt} \right)^{-1} = V \beta_V + S \beta_S \quad (33)$$

where β_S represents the surface buffering capacity and S the surface area present. This equation again

is to be considered to be the defining equation for the surface-buffering capacity. Many surfaces contain reactive groups that bind or release protons; in particular, most polymer surfaces contain carboxylic acid, amine, or OH groups, and therefore buffer pH to some extent.

Equation (33) can be rewritten as:

$$\frac{dpH}{dt} = \frac{R}{V \beta_V + S \beta_S} \quad (34)$$

This expression directly relates the rate of change of the pH in the volume to the rate of the chemical reaction that causes the pH change. To obtain the function that describes how the pH varies with time, eq (34) needs to be integrated. In general, both R and β_V depend on pH, and thus dpH/dt will not be constant. However, in a region where the right hand side of (34) depends only weakly on pH, the pH will vary linearly with time.

We will now consider the value of the volumetric buffer capacity β_V . The simplest case to analyze is the one where buffering comes from a single acid/base pair in solution. It is well known from elementary physical chemistry that the pH of a solution containing such a buffer pair is given by:

$$pH = PK + \log_{10} \left(\frac{[\text{base}]}{[\text{acid}]}} \right) = pK + \log_{10} \left(\frac{[\text{base}]}{c - [\text{base}]}} \right) \quad (35)$$

where K is the equilibrium constant of the reaction between the acid and basic forms of the buffer pair, $[\text{acid}]$ and $[\text{base}]$ are the concentrations of these forms, and c is the total buffer concentration. This can be rewritten as:

$$\frac{c - [\text{base}]}{[\text{base}]} = \exp(x) \quad (36)$$

where $x = 2.303(pK - pH)$. If a certain number dn of moles of OH⁻ ions are added to the solution, they will react with an equal number of moles of the acid form of the buffer pair to produce $Vd([\text{base}])$ moles of the basic form. By referring to the definition (36) of the buffer capacity above, it can be seen that:

$$\beta_V = -2.303 \frac{d[\text{base}]}{dx} = 2.303 c \frac{e^x}{(1 + e^x)^2} \quad (37)$$

This expression for the volumetric buffer capacity of a buffer pair tells us several important things. First, as could be expected, the buffer capacity is proportional to buffer concentration. Second, the magnitude of the buffer capacity does not depend on the nature of the buffer; only the pH at which the buffering occurs depends on the buffer pK through x . When the pH equals the pK of the buffer pair, the buffer capacity is maximal:

$$\beta_V(\max) = 0.576 c \quad (38)$$

Therefore, apart from a shift of the maximum along the pH axis, the curve of buffer capacity versus pH does not depend on the nature of the buffer.

These calculations of volume buffer capacity can now be used to calculate the pH/time curves, provided two assumptions are made: first, that surface buffering is negligible, and second that R does not depend on pH. Substitution of eq (37) into eq (34) then yields a differential equation, which can be readily integrated to give

$$t^* = \frac{R}{Vc} t = \left[\frac{1}{1 + \exp(x)} - \frac{1}{1 + \exp(x_0)} \right] \quad (39)$$

where x_0 is the value of x at $t = 0$, and t^* is a dimensionless time that incorporates the effects of chemical generation rate, chamber volume and buffer concentration. Figure 49 shows plots of this equation for various values of x_0 . The curves all go through an inflexion point at $\text{pH} = \text{pK}$; far away from pK the slope of the curves increases because the buffer capacity is less. For certain values of x_0 , the curve has a large region of linearity close to $\text{pH} = \text{pK}$. If we choose the initial pH to be about 0.5 units below pK, the curve is linear up to 0.5 pH units above pK.

We can calculate the average slope of the curve in this linear region, and derive the approximation:

$$\frac{dpH}{st} = 1.8 \frac{R}{cV} \quad (40)$$

and by introducing the sensitivity a of the EIS device, which is used to measure the pH changes, we can restate this in terms of the measured voltage output

$$\frac{d\psi}{dt} = 1.8 \frac{\alpha A E}{cV} \quad (41)$$

where y is the measured signal from the pH sensor.

In this equation we have also substituted the expression (3.5) for the rate of proton generation in the case of the presence of an enzyme. The sensitivity a needed in this equation is the immediate response of the silicon nitride surface to pH changes, which has been found to be typically 56 mV/pH for our samples (see sec. 2.2.3). The factor $(1.8aA/cV)$ is the sensitivity of the system to the presence of urease. Using values typical for the immunoassay system to be described below, namely: $A = 8 \times 10^{-3}$ mole/g $\diamond$ s, $a = 0.056$ V/pH, $c = 0.01$ mole/liter, and $V = 6.4 \times 10^{-7}$ liter, it can be calculated that

$$1.8 \frac{\alpha A}{cV} = 1.26 \times 10^5 \text{ V}/(\text{s}\cdot\text{g}). \quad (42)$$

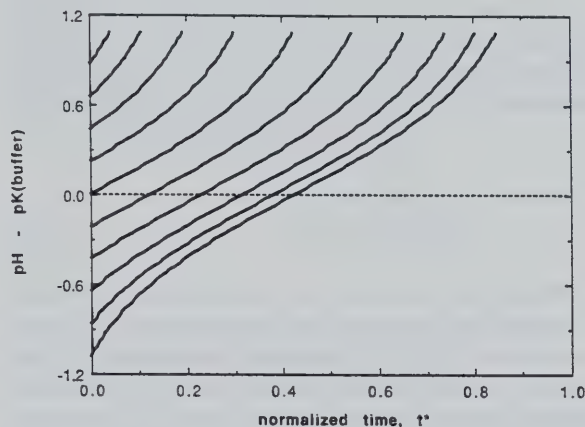


Figure 49. Theoretical pH/time curve for a pH-changing enzyme such as urease in a solution containing one pH buffer at $\text{pH} = \text{pK}$.

The lowest detectable dy/dt depends on how much settling time is allowed since the pH changes. Figure 47, in the previous section shows the drift rate after a pH step for silicon nitride. The initial drift rate is about 4×10^{-6} V/s. If we assume that there is no waiting time because the last pH change, the lowest rate distinguishable from zero can be estimated to be 5×10^{-6} V/s. By waiting longer between measurements, this value could be lowered, but the wait could be considerable. According to figure 47 it takes 17 minutes for the drift rate to go below 10^{-6} V/s. Assuming a rate of 5×10^{-6} V/s, and by substituting the value from eq (42) into eq (41), it can be calculated that the lowest detectable amount of urease is 40 picogram. This corresponds to 4.4×10^7 molecules. However, it is feasible to lower both c and V by another factor of 5 to 10. The detection limit could then be lowered to the 10^5 molecule range. The most sensi-

tive detection we have achieved was reported by Hafeman et al. [6] as 6×10^5 molecules, a mass of 0.54 picogram. It thus appears that this method enables the quantitation of analytes in the subpicogram range. The key to achieving such high sensitivity is the very low volume of the chamber and the low buffer capacity.

In most of the derivations above, the effect of surface buffering has been neglected. When a porous membrane with a high surface area is used, this is not necessarily justified. By starting from eq (37), the previous reasoning can be repeated without neglecting surface buffering, with the result:

$$\left(\frac{d\psi}{dt}\right)^{-1} = \left(1.8 \frac{\alpha A}{V}\right)^{-1} \frac{c}{E} + \frac{S\beta_s}{\alpha A E} \quad (43)$$

The surface area S and the surface buffer capacity β_s are not usually known. It is then most convenient to group these two parameters into a single unknown, the surface buffering expressed as an equivalent volume buffer concentration c_s :

$$c + c_s = \left(1.8 \frac{\alpha A}{V}\right)^{-1} E \left(\frac{d\psi}{dt}\right)^{-1} \quad (44)$$

From this equation follows a method of experimentally determining c_s which is given in detail in reference [99]. It consists of measuring the response per amount of urease present at different buffer concentrations, and plotting c as a function of $1/\text{signal}$. For the nitrocellulose membranes we use (about 100 μm thick and 0.45 μm pore size) c_s is found to be about 0.0023 mole/liter. By using a volume buffer concentration of 0.01 mole/liter, the effect of surface buffering can be eliminated. Thus, the calculations based on eq (42) are valid.

3.2.3 Concentration of Enzymes in a Small Volume As noted above, in the immunoassay system described in this section the enzyme is used as a label to detect another species. The exact method to do this has not yet been discussed. Another issue is that sensitive detection requires a small volume. The question thus arises of how to concentrate all the label enzymes into this small volume.

Both these issues can be resolved by using an antigen/antibody binding reaction, which results in the formation of an antibody-antigen complex. More specifically, assume we want to detect an analyte X , and that we have two different types of antibodies to X available. (Another possibility is a single antibody, but that can bind to several different sites of molecule X). We now need to attach other molecules to these antibodies, a process that

is conjugation. One of the conjugates is an antibody-label enzyme complex, with the label enzyme being urease. The other conjugate is used for capturing the immunocomplex on the surface of a porous membrane. Such a membrane has the advantages of possessing sufficient surface area for capture in a small volume.

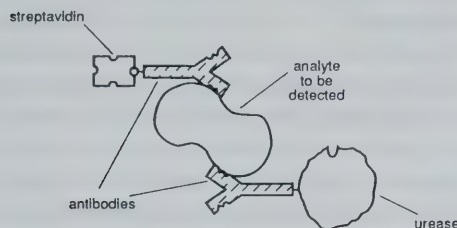
To ensure that a very high fraction of the label enzymes is attached to the surface of the membrane, some form of active capture of the immunocomplexes is needed. This will be described in this section. Active capture means that a stronger and more irreversible chemical reaction than the adsorption of macromolecules such as proteins on to solid surfaces is relied on for attaching the immunocomplexes. The important aspect is irreversibility, because a wash step is needed to remove all the enzyme conjugates that have not found a molecule X to bind to.

The capture is done by using a pair of molecules that bind to each other with very high affinity. A common example is biotin/avidin system. Biotin is a water-soluble vitamin, and avidin is a protein in egg whites. Avidin is a tetramer: it is a protein composed of four identical subunits, each of that has a molecular weight of 15,000. Each avidin subunit is a binding site for biotin, so that up to four biotin molecules may be bound to one avidin. The equilibrium constant for the dissociation reaction of the avidin/biotin complex is 10^{-15} M. This is so low that effectively the bound complex between the two never comes loose. A dissociation constant of 10^{-15} M implies that for concentrations in the millimolar range only about one in a million complexes will dissociate. Possible methods of using the biotin/avidin system for capture are to make a biotin/antibody conjugate and to attach avidin to the surface, or vice versa: make an avidin/antibody conjugate and attach biotin to the surface.

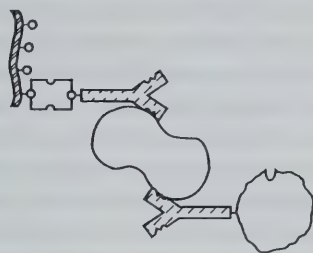
3.2.4 Description of an Immunoassay System We have now described enough of the concepts in a silicon sensor immunoassay that the whole process can be described in detail. A schematic of the process is shown in figure 50. The steps involved can be listed as follows:

1. Assume that analyte X is to be detected and that two antibodies to X are available. The first step is to make conjugates with these antibodies: one is an anti- X /urease conjugate; the other is an anti- X /biotin conjugate.
2. Add these conjugates to the sample to be analyzed, together with some free avidin, and incubate (i.e., wait a given time at a temperature of

Formation of Immunocomplexes in Solution Phase



Capture by Filtration through Biotinylated Membrane



Detection of Urease in Microvolume Reaction Chamber

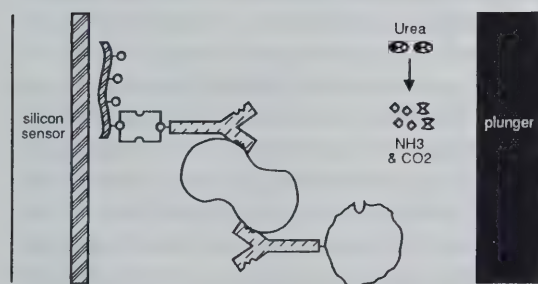


Figure 50. Schematic of a silicon sensor immunoassay using filtration capture, and detection in a microvolume chamber.

typically around 37 °C). This will form the immunocomplexes of urease/anti-X/X/anti-X/biotin/avidin.

3. Filter the solution with these immunocomplexes through a membrane that has biotin bound to its surface. By choosing a sufficiently slow filtering rate (around 5 min) and membranes with small pore size (in the order of a micrometer or less) a capture efficiency of better than 90% can be attained.
4. Filter through a wash solution (typically physiological saline with small amounts of detergent added) to remove all the anti-X/urease conjugates that are not bound to an X molecule.
5. Place the membrane in a low-volume chamber in that one wall is a silicon sensor. The chamber is filled with a solution similar to the wash solu-

tion, but with urea added. The urease in the membrane therefore causes the pH to rise, as described in section 3.2.1. The rate of change of the pH vs. time is proportional to the amount of X present.

An example of an assay we have developed along these lines is a measurement of total DNA with picogram sensitivity [100,101]. Both the hardware and the reagents needed for carrying out assays such as described here are available from Molecular Devices Corporation. One silicon LAPS chip has nine active areas on it, enabling nine rates to be read simultaneously from one membrane: eight channels and one reference.

3.2.5 Sensitivity of the Measurement System

In section 3.2.1, the sensitivity to urease of this immunoassay system was derived. However, what we really want to know is sensitivity and detection limits to the actual quantity we want to detect, that is called X. This can easily be described starting from the sensitivity to urease.

Let's assume that n urease molecules are linked to every X molecule. There are two ways in which values of n higher than one can be achieved: more than one anti-X/urease conjugate could bind to each X molecule, and each conjugate could contain more than one urease. In both cases, the numbers should be seen as averages taken over the entire population of X molecules and conjugates. There will always be individual variations, particularly in the making of conjugates where a range of different products is obtained. We can write the following relationship between the mass of X molecules and the mass of urease molecules present in the conjugate bound to them:

$$x = \frac{M_x}{nM_u} E \quad (45)$$

in which M_x and M_u are the molecular weights of X and urease, respectively. This can now be introduced into eq (44) to yield:

$$\frac{d\Psi}{dt} = 1.8 \frac{\alpha A' n}{v M_x} x \quad (46)$$

which is the description of the sensitivity of the assay to the mass of X molecules. In this equation, $A' = \alpha M_u$ represents the molecular activity or turnover number of the enzyme. Equation (46) is valid in the assumption that surface buffering is negligible, that is true if the bulk buffer concentration is at least 10 mM. If surface buffering cannot

be neglected, eq (46) must be generalized as shown above.

Equation (46) shows that the sensitivity of the assay increases linearly with the turnover number of the enzyme used. In the case of urease, the turnover number we have measured at room temperature is $4.8 \times 10^5 \times (8 \times 10^{-3}) = 3840$ per second. We can therefore define a new system sensitivity constant, similar to eq (42), but using A' instead of A :

$$1.8 \frac{\alpha A'}{cV} = 6.048 \times 10^{10} \text{ V/(s}\cdot\text{mol)} \quad (47)$$

To illustrate the meaning of this sensitivity constant, let's assume that the molecule being detected has a molecular weight of 10^5 g/mol, and that $n = 1$. Then, assuming as before that the minimum detectable signal is 5×10^{-6} V/s, it can be calculated that the detection limit for X is:

$$x = 8.3 \text{ pg.} \quad (48)$$

The assumptions here are fairly conservative, because a molecular weight of 10^5 is rather large, and we have not assumed any signal amplification that would make n larger than one. In fact, with small molecules the sensitivity should be higher because then there are more molecules for a given mass. When a molecule is larger, the assay will be less sensitive, unless a way is found to increase n beyond one. This is conceivable, because with a larger target molecule there is space to attach more than one antibody or other binding molecule.

Another issue to bear in mind is that in practice the limitation to the sensitivity is often not the one implied by eq (48), but rather a background signal caused by nonspecific binding of urease conjugates to the membrane. There are a number of techniques known in the field of immunoassays to reduce nonspecific binding [102], and these should be employed to enhance sensitivity.

3.2.6 Conclusions We have illustrated how an immunoassay based on a silicon field effect sensor can yield picogram sensitivities. The key characteristic of the system that enables such a high sensitivity to be obtained is the low detection volume. The low volume, in turn, is made possible by (1) the flatness of the silicon sensor, (2) the absence of bonding wires to the frontside of the sensor, and (3) the concentration of the immunocomplexes on a thin membrane by specific capture.

Measurement methods must be compared not only by their sensitivity, but also by the accuracy of

the quantitation they permit. Some biological methods exist that are highly sensitive but only qualitative.

The price to be paid for obtaining a high sensitivity is that the assay requires some sample manipulation. It does not work simply by immersing a sensing probe of some kind into a sample and reading a result. In the language used in the field of immunoassays, it is a heterogeneous assay and not a homogeneous one. It is a general observation that heterogeneous assays are superior, and few homogeneous assays are practically used [102]. An important aspect of the filtration and wash step is that it separates the desired analyte from all other constituents of the sample. It is the presence of these other constituents that often limits the values of homogeneous immunoassays using optical methods such as surface plasmon resonance or mass-sensitive methods involving mechanical resonance such as surface acoustic waves. A biological sample usually contains a large amount of protein, that will adsorb on practically any surface. This nonspecifically bound protein will dominate the mass change or change of optical properties at the interface. A typical sensitivity limit for homogeneous immunoassays is in the nanogram range [102].

A heterogeneous assay involves more work on the part of the user. In the method described here, the sample must be combined with the conjugates that bind to it, incubated, filtered through the membrane, wash solution and must be filtered through, and the membrane must be transferred to the reading chamber. A challenge in the further development of highly sensitive heterogeneous immunoassays is to automate these steps, so as to make them invisible to the user. Ideally, all that should be required is to place a sample in the instrument. Further work, and possibly new concepts, will be required to make this a reality.

4. EIS Sensors as Monitors of Cellular Metabolism

4.1 Background

The idea of using living cells as components of a biosensor system is not new. One example is the use of a tissue slice as a component of an ion-selective electrode. The tissue slice is used as a chemical transducer that translates the concentration of an unknown into a product that can be detected with the ion-selective electrode. An even more innovative concept has been presented recently by Lundstrom et al. [103]: the fish scale biosensor. Scales

from a certain species of fish have the ability of changing pigmentation in response to norepinephrine or other catecholamines. The response is detected optically, by placing the fish scale in a simple photometer system. In this way, the activity of substances on the adrenaline receptors in the fish scales can be measured. The particularity of this approach is that the fish scale is not used as a chemical transducer, but as a biochemical system with receptors to certain substances and as a response mechanism.

In this section, I will describe a system with certain similarities to the fish scale biosensors, but with more general applications. It detects the metabolism of individual cells that have been cultured or taken from organisms. The system is a biosensor in the sense that it uses living cells to measure quantities of biological interest. In our system, a silicon pH detector is used to determine the metabolic rate of a relatively small number of cells by measuring the rate at which they acidify their environment. The cells have to be retained in a small volume in order to detect their metabolism in this fashion. Therefore, the silicon chip used to detect pH must be part of a microvolume chamber in that the cells are immobilized. The detector we use is the LAPS (light-addressable potentiometric sensor) [6], that is particularly suitable for this application.

We have found that in addition to performing the chemical-detection function, the silicon chip can accomplish mechanical and fluidic functions, through the use of micromachining techniques that have been developed recently for the fabrication of many types of solid-state sensors or actuators. Two functions accomplished through micromachining are accurate determination of the volume of the chamber in that the cells are located, and entrapment of nonadherent cells.

The technology described here constitutes part of a system for carrying out rapid and sensitive bioassays. We have called this system a microphysiometer [104], because it measures metabolic rates. Examples of some of its possible applications are *in vitro* toxicology using human keratinocytes [104]; determination of the sensitivity of tumor cell lines to chemotherapeutic agents [104]; investigation of antiviral agents [104]; and screening for therapeutic drugs by detecting ligand-receptor binding [104–108].

4.2 Rapid Overview of Cellular Metabolism

Living cells are entities in that many chemical reactions are maintained in steady-state positions

far removed from equilibrium. An example is the concentration of cations such as sodium, potassium, and calcium, which is usually very different inside and outside the cell membrane. To maintain these differences, ion channels act as pumps to transport ions against a concentration gradient. Transport against a concentration or potential gradient requires energy because it results in a system with more energy and less entropy. Therefore, every case of a non-equilibrium steady state requires a constant energy output to maintain it.

Cells use energy for a variety of purposes, such as mechanical motion or deformation, movement of chemical compounds against concentration gradients, and the synthesis of compounds. Living organisms can derive energy from two sources: light from the sun or the consumption of energy-rich compounds coming directly or indirectly from other organisms. The first category consists of plant life, and the second category consists of all other life.

Nonplant life has two general types of metabolic pathways: anabolic, in that compounds are synthesized and energy is used up, and catabolic, in that compounds are broken down and energy is liberated. This energy is stored in the form of adenosine triphosphate (ATP), that is the universal energy currency in biology. Reactions that require energy input obtain it by hydrolysis of ATP to adenosine diphosphate (ADP), that liberates 7.3 kcal/mol of energy. The catabolic pathways that generate energy do so in the form of increasing ATP concentrations. A typical total concentration of the adenosine phosphate species in a cell is 2 to 10 mM.

Two types of catabolic pathways can generate energy for a cell. The first type is glycolysis. It occurs in the absence of oxygen (anaerobic conditions): the starting material is glucose, which is oxidized to lactic acid. In the presence of oxygen, a more complete metabolic breakdown of glucose occurs, and CO₂ is the ultimate product. This carbon dioxide dissolves in water, and some of it is hydrolyzed into carbonic acid. Thus, in both cases the end result of an energy-yielding metabolic pathway is an acidic substance. The metabolism of living cells therefore always acidifies the environment. This is the key feature that allows the measurement of the metabolic activity of cells.

Many factors can affect the metabolism of a cell. The well-being of a cell requires a certain minimal metabolic rate, and rates below that indicate that the cells are unwell or dying. This is important in *in-vitro* toxicology applications. From a more gen-

eral point of view, because cellular activity requires energy and therefore ATP consumption, the metabolic activity that replenishes the ATP supply is sensitive to virtually anything that happens in a cell.

Receptors constitute a very important way in that cells respond to their environment. Biological molecules such as hormones and drugs evoke their effects by interacting with certain specific “receptors” in the body. The presence of receptors that are specifically sensitive to the presence of certain classes of scientists was deduced by scientists long before their detailed biochemical nature was discovered. It has long been known that substances that bind to receptors fall in two categories: agonists, that bind to a receptor and elicit a biological response, and antagonists, that also bind but do not elicit a response. A classic example of an agonist is nicotine, which stimulates the contraction of frog muscle, through a receptor now known as the nicotinic acetylcholine receptor. An antagonist for this receptor is curare, a poison that causes muscle paralysis. Receptors are now known to be large proteins embedded in the outer cell membrane. Part of these receptor proteins is located in the extra-cellular environment, and part is in the cytoplasm (the inside of a cell). The outside part binds selectively to a ligand; if the substance is an agonist, the binding will trigger events inside the cell. If the substance is an antagonist, it will bind without triggering these effects.

The effect of the events triggered in a cell by an agonist typically increase the metabolic rate. This increase can be considerable—typically on the order of 50%, and over 100% in some cases. Thus, in a system capable of sensitively and continuously measuring metabolic rate, the binding of an agonist to a receptor on a cell membrane can be detected. Modern drug discovery relies heavily on finding specific agonists or antagonists to a well-defined receptor. An example of this are the beta-blocker drugs against heart disease, which are specific antagonists to the beta-adrenergic receptor. Clearly, the measurement system of cellular metabolism described here could be useful in drug discovery.

4.3 Measurement of the Metabolic Rate

In order to survive, the cells have to be bathed in a relatively fresh culture medium. This medium supplies the cells with nutrients and keeps the acidity of the environment in a tolerable range. The medium must be replenished constantly to bring fresh nutrients and remove the acid by products of

cellular metabolism. A flow of medium is therefore required on average. However, when cells are placed in a microvolume chamber through that culture medium is flowing (at a rate of typically 100 $\mu\text{L}/\text{min}$), the chamber pH is determined by that of the medium. Thus, metabolic acidification cannot be detected while the medium is flowing. To measure cell metabolism, the medium flow must be periodically interrupted. As the cells consume glucose, they excrete acidic metabolic products (carbon dioxide and lactic acid, produced by aerobic respiration and glycolysis, respectively). The cells therefore act as a source of protons, that will change the pH at a rate depending on the cavity volume and the pH-buffering capacity of the medium. This situation is similar to the one where an enzyme catalyzes a reaction that changes the pH in a small volume, and that was analyzed in the previous section. In fact, eq (40) still applies, but with the rate of acidification R now being given by:

$$R = N_{\text{cell}} R_{\text{cell}} \quad (49)$$

where N_{cell} is the number of cells in the chamber, and R_{cell} is the rate of acidification caused by a single cell. The whole analysis in section 3 still applies with this new source of pH change, in particular the importance of both the bulk and the surface buffer capacity. In order to enhance sensitivity, we use nutrient media with reduced buffer capacity compared to the usual formulations. Provided the proper pH is maintained, buffer concentration does not affect the cells.

The previous equation implicitly assumes that only one type of cells is present. It can be generalized to the case that several types of cell are present:

$$R = \sum_i N_{\text{cell}}^i R_{\text{cell}}^i \quad (50)$$

These different types can be present intentionally or unintentionally. The unintentional case presents itself when a cell culture is impure or contaminated because the measured metabolic rate R depends on all cells present, impure cultures will yield erroneous results. Examples of contaminants are bacteria or mycoplasmas (i.e., pathogens much smaller than bacteria and hard to detect). An example of an intentional diversity of cell type is a culture of cells infected by a virus. In that case, the two populations are the infected and the uninfected cells. As the infection spreads, the total metabolic rate will tend to drop below the level it reaches without the virus. This has obvious applications, as is discussed below.

In order to create an experiment with unambiguous interpretation, the number of variables that affect the metabolic rate must be reduced to one, or two at the most. In most cases, this requires ensuring that only one type of cell is present, so that the only two remaining variables are the number of cells and their metabolic rate. Usually it is possible to keep one of those two constant, to some approximation. By not changing the solution that flows past the chamber, for instance, R_{cell} is kept constant. Then, the measured R is an indicator of the evolution of the total number of cells. If the cells are bacteria, that multiply exponentially with a characteristic doubling time, this doubling time can be measured. The other situation arises when the number of cells does not change. This can occur in many situations, depending on the cell type. If the cells are neurons, for instance, they will not divide. Adherent cells that have spread out to cover a surface with a confluent monolayer will also not multiply further. When the number of cells is at least approximately constant, it is possible to monitor the effect of the addition of a test compound to the flow medium on the metabolic rate of a single cell, R_{cell} .

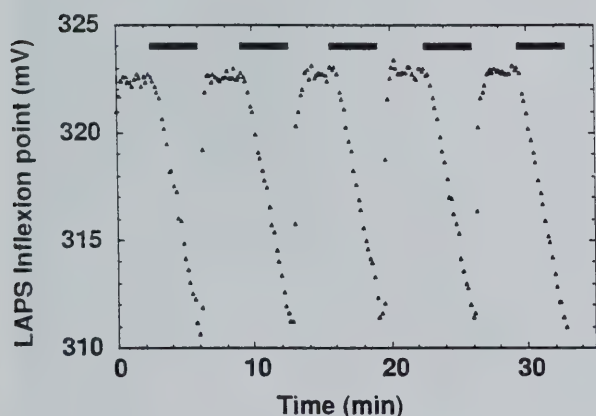


Figure 51. Example of metabolic rate detection using a LAPS device, and on/off switching of medium flow.

The flow pattern needed consists of an on-phase to renew the solution in the chamber and to reset the pH to that of the medium. The on-phase is followed by an off-phase during which the rate of acidification is measured with the pH sensor. This flow on/flow off cycle is repeated within a period of 50 to 300 seconds. A typical chamber has dimensions approximately 100 μm high, 3 mm wide and 12 mm long (volume 3.6 μL); the volume used for pH detection, however, is about 100 nL, and contains typically about 1000 cells. Figure 51 shows an

example of metabolic rate detection, with a typical rate of 60 $\mu\text{V/s}$, or 0.06 pH units/min.

4.4 Microvolume Flow Chambers for Cells

The flatness of the LAPS sensor enables it to be used as one wall of a microvolume flow chamber. There are many possible methods for building such chambers. One technique that we have found very useful is to use a piece of thin glass (a microscope cover slip) as the opposite wall of the chamber. The thickness of the chamber can then be determined by a spacer, such as a teflon sheet at the edges. Glass cover slips have the advantages of being cheap and allowing the observation of the cells inside the chamber. The problems that the mechanical design of the cell chamber has to solve are the sealing of the fluidic path, and the provision of entry and exit ports for the fluid flow. Figure 52 shows a cross-sectional diagram of a flow chamber and incorporated LAPS device for cells adhering to a glass cover slip.

An entirely machined cell chamber will have a volume determined mainly by the accuracy of the machining and the thickness of the spacer material. A typical accuracy of machining is around 10 μm in hard plastics; more accuracy can be obtained in metals, but that then raises problems of electrical insulation. Because we are aiming for chambers about 100 μm thick, reproducibility of the volume is an issue. For any one chamber, knowledge of the exact volume is not critical, but if two chambers are being compared to each other, for instance, for an experiment with a simultaneous control, the reproducibility of the volume is important.

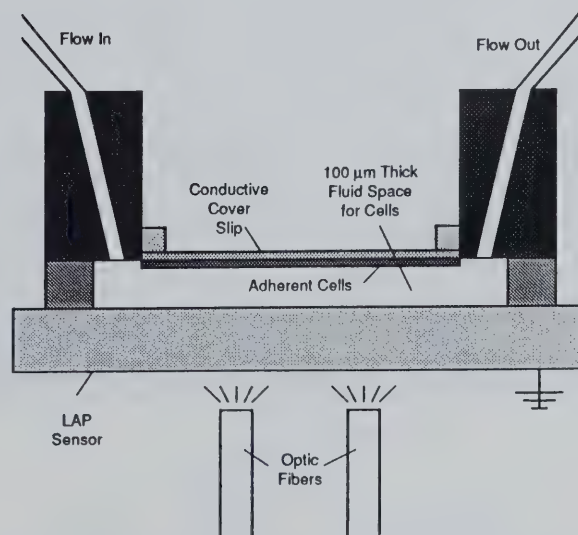


Figure 52. Cross-sectional diagram of a flow chamber for cells.

One obvious method to improve the accuracy of the chamber volume is to replace the spacer layer, or the machining of a chamber in a piece of plastic, by micromachining the piece of silicon in that the LAPS sensor is made. This application of silicon micromachining thus consists of the etching of channels in the silicon to serve as the fluidic pathways and accurately defining the volume of the microvolume chambers. The chamber is again made by placing a flat plate, such as a glass cover slip, with entry/exit ports over the etched silicon. The main requirement for this etch is the creation of a sufficiently smooth bottom surface. We have found this can be achieved with a wet anisotropic etch of the type commonly used in silicon micromachining. This step is carried out at the beginning of the process used to make a LAPS sensor (see sec. 2). In essence, the anisotropic etch sculpts a shape in the silicon starting material that is then used to make a LAPS in exactly the same fashion as on a flat piece of silicon. In the basic process sequence given in section 2, this step is added between steps 2 and 3. If a masking oxide is used for the etch and is not removed, the result is that the active area is the same as the area that is etched down. Figure 53 is a picture of a sensor chip with a fluidics pattern etched into it. The dimensions of the etched-down area are relatively large (about 13×7 mm), that means that the 55° angle at the edges has little impact.

The anisotropy of the etch is used here to achieve a very smooth bottom surface. By starting with a polished (100) surface, and using an etch that attacks that surface very uniformly, most of that polish can be maintained even when the etch depth is as much as $100\ \mu\text{m}$. However, the anisotropy means that the pattern etched into the silicon will not be the same as the one on the mask in most cases. The (100) directions will be favored, and sides with this orientation will appear or increase in length relative to the pattern on the mask. The discrepancies will not exceed the order of magnitude of the depth of the etch, however, and because the flow chamber patterns are on the order of one millimeter in dimension, these variations can be tolerated. Furthermore, it is possible to predict them and compensate for them. A smooth and very uniform isotropic etch might be preferable in this application, but we have not yet perfected such a process.

4.5 Methods for Cell Entrapment

The method used for retaining the cells in the microvolume chamber depends on the cell type.

Some cells are adherent, that means they will stick to a solid surface on contact. An example is the human skin cells used for the in-vitro toxicology work described below. The most convenient way to culture these cells is to grow a monolayer of them on a solid support. Other cells are nonadherent. They float around in the medium, but do not attach themselves to anything. Using non-adherent cells is thus more of a challenge, because a method must be found to retain them in a chamber and still allow medium flow to pass through the chamber. Some cells are mostly non-adherent in the sense that they do not form a well-defined monolayer on as solid surface, but many still have a little stickiness. This can help retain them, depending on the method chosen.

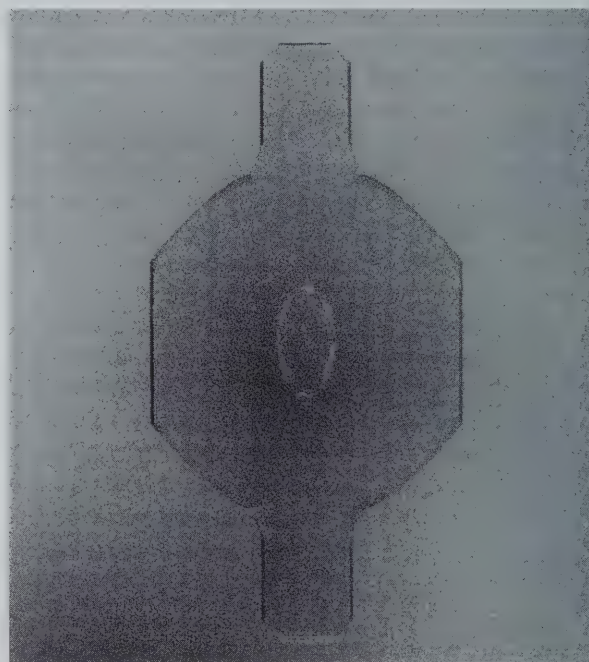


Figure 53. Picture of a sensor chip with a fluidics pattern etched into it.

4.5.1 Methods for Adherent Cells If the cells are adherent, a confluent monolayer can be grown on a solid support. Two surfaces can be used for this: the silicon sensor itself, or the glass cover slip that is used as the chamber wall opposite the silicon. In either case, a monolayer of cells is present in the same chamber volume, so the metabolic rate determinations should be the same. However, if the cells are grown on the silicon, there is a possibility that further complications could arise, due to the presence of a layer of cells between the silicon pH sensor and the bulk of the solution. First, if there is an electrical potential between the top and

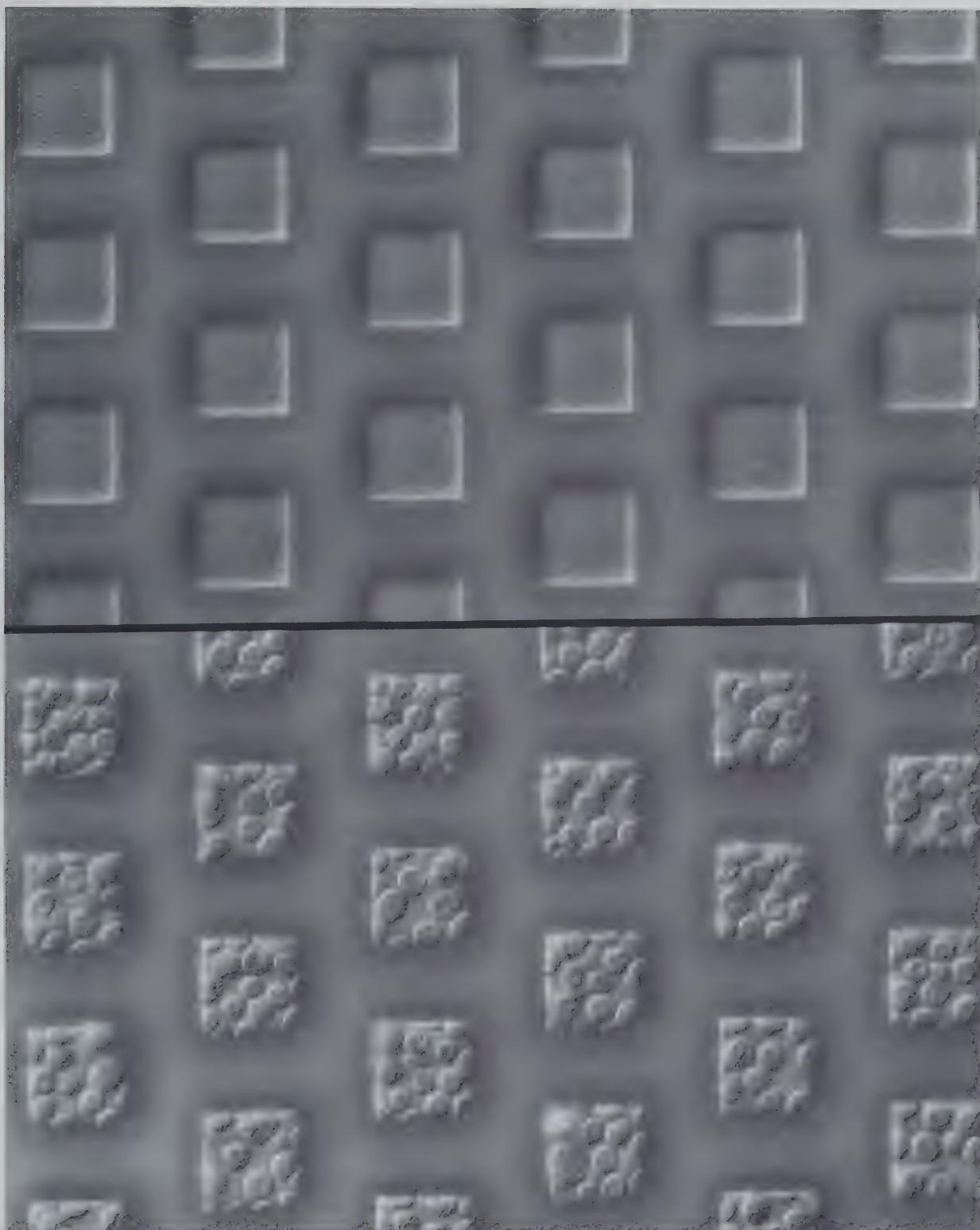


Figure 54. Plasma etched cavities in a LAPS device before and after filling with cells.

the bottom of the cell layer, it will affect the measurement. Some cells can create such potential differences. Second, the pH above and below the cell layer might be different. Both these effects depend on the conductivity of the cell layer, for protons and for electric charge, respectively. If the layer is sufficiently confluent that these conductivities are not high, then these effects could change the measurement. This is the reason that in general we avoid such possible difficulties by growing the cells on a glass cover slip.

4.5.2 Methods for Nonadherent Cells For nonadherent cells, some method of retention must be found to avoid the cells being swept away with the medium flow. Many possibilities exist. Some of the constraints are that the retention mechanism must not add too much surface buffering capacity, and that the cells must not be mechanically damaged. We have found that an effective and gentle method is to allow the cells to sediment by gravity into a collection of wells. The most effective way to do this is to etch these cavities in the surface of the silicon sensor itself [104,109].

The fabrication of cavities for nonadherent cells requires the application of silicon micromachining techniques. It is necessary that cells remain in the cavities even when a sufficient flow of nutrient medium goes through the chamber. This implies that the flow lines cannot penetrate very much into the cavity itself. Clearly, this requires deep cavities with walls close to vertical. The size of the cavities must be larger than the typical dimensions of a cell, that are on the order of 10 to 20 μm . Our typical cavities are 50- μm squares, although we have experimented with various other patterns, such as triangles and hexagons. The cavity depth must be at least equal to the side of the square, so that the etched volume is roughly a cube.

To meet these requirements while still retaining the (100) orientation of the starting material, we chose anisotropic plasma etching. This method was described by Zdeblick et al. [110,111]; the gases are SF_6 and C_2ClF_5 in a 1:1 ratio. The cavity depth was measured to be about 50 μm , and the angle of the walls was between 85° and 90° . As in the case of the wet etch, the etch was done after the active area definition and before gate oxide growth. Therefore, only the sides and bottoms of the cavities are active pH-sensing areas. This means that the inside of each cavity, with a volume of 0.125 nl, acts as an individual pH sensor. The photocurrent generated in the LAPS device is the sum of the the contributions from a 1- mm^2 array of about 180 of these cavities. The illumination from the backside with

modulated light thus acts as a virtual backside contact for potentiometric sensors, and enables all sensors in the area covered by the light beam to be accessed simultaneously.

The method for filling the cavities consists of filling the entire chamber with a suspension of cells, and allowing them to settle by gravity into the wells. A slow flow of liquid (linear velocities 20 to 100- $\mu\text{m/s}$) then sweeps away the cells that are not trapped in the bottom of a well. Figure 54 is a composite photograph showing a set of cavities before and after this filling process.

The pattern of laminar flow over a deep rectangular cavity is relatively well understood, both theoretically [112] and experimentally [113]. Figure 55 shows the flow pattern. The most important aspect is the existence of separating flow line, that penetrates slightly into the cavity. Mass transport across this line is by diffusion only. Below this flow line are one or more vortices, depending on the depth of the cavity. For a 1:1 aspect ratio, there is room for only one vortex. At about a 2:1 depth-to-width aspect ratio, a second, much slower, vortex appears. In this context, the important point is that cells packed in below the separating flow line will not be swept out of the cavities by a slow flow in the laminar range. The vortex will only agitate cells within a cavity, as we have in fact observed.

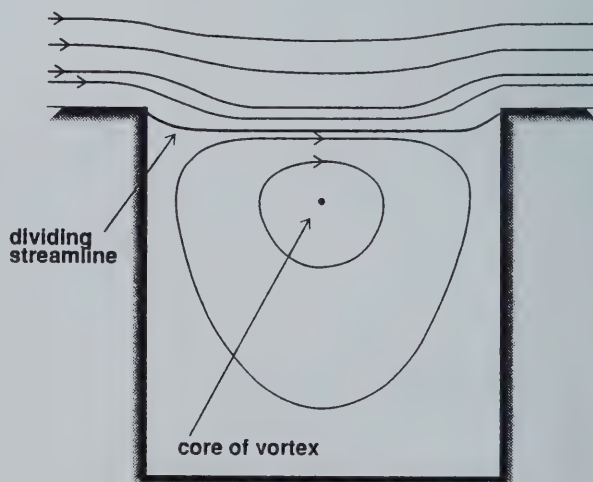


Figure 55. Flow pattern for laminar flow over a deep rectangular cavity at very low Reynolds number (in the order of 1).

4.6 Examples of Applications

The strengths of a microphysiometer using a silicon detector for carrying out a bioassay can be summarized as follows:

1. A low number of cells is required. Only about a thousand cells are in the active region where metabolism is measured.
2. The response is rapid, down to about a minute at present (although special data manipulation can uncover even faster responses, see [105]).
3. The system is general, in that any type of cell can be used, and metabolism is a very sensitive indicator of just about any change that occurs in the cell.
4. It is relatively easy to design a system that allows for parallelism, with for instance eight separate cell cavities and fluid flows.

The possible range of applications of sensitive detection of metabolic changes is tremendous. In the following sections I will describe a few of them, in order to give some idea of the possibilities. It is highly likely that further research will uncover many more applications.

4.6.1 In Vitro Toxicology One fertile area of applications is in vitro toxicology. The aim of toxicology is to understand the damage done to the human body by exposure to toxic agents of any kind. One traditional way of testing compounds for their toxicity is to use animals for testing. Measures of toxicity for ingested or injected substances such as LD_{50} are determined in that way. For certain areas of toxicology, it would be desirable to obtain information more directly applicable to the human body, rather than to animals such as rats or mice. This is in addition to the general desire to limit the use of animal testing as much as possible.

One example of a case where animal testing has clear drawbacks is the assessment of the skin irritancy of a compound. The animal test method used for this is the Draize test, that consists of placing the compounds in the eyes of rabbits, and assessing the degree of irritation observed. Some of the drawbacks of this test are: first, rabbit eyes are not exactly the same as human skin or eyes; second, the compounds tested are often not drugs but consumer products for that the ethical justification for animal testing is weaker; and third, the test is qualitative, unreliable, and gives no information about the mechanism of the irritancy.

We have worked on a testing method that uses human skin cells (keratinocytes). These cells are cultured and coated on a glass cover slip on which they form a near-confluent monolayer. Then they are placed in a chamber with a silicon detector to measure the metabolic rate. Once the baseline metabolic rate is determined, a certain concentration of the substance under test can be injected for

a fixed period. This is done first at a very low concentration, where no effect on metabolism is seen, and then at progressively higher concentrations until the metabolic rate of the cells begins to fall. Eventually, the rate drops to near zero, indicating that most cells have been killed. The interpolated concentration where the metabolic rate is 50% of the original baseline is called MRD_{50} (metabolic rate decrease). This is a quantitative indication of the degree of toxicity of a compound to human skin cells (see fig. 56). We have determined the MRD_{50} for a number of compounds and compared them to the known Draize test scores. A good correlation is found, especially because the Draize test scores are not truly quantitative.

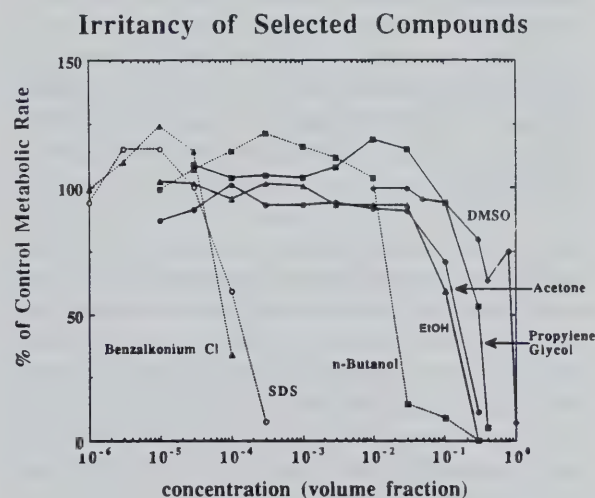


Figure 56. Correlation between MRD_{50} numbers obtained with a microphysiometer and Draize test scores.

Another useful aspect of the microphysiometer test is that it yields more information. It is possible to track the evolution of metabolism with time after the administration of a toxic compound and to observe the recovery from a toxic insult. One test, for instance, consisted of exposing cells for a certain period to ethanol and to DMSO, each at the concentration of its MRD_{50} , and then tracking the fate of the cells. In both cases, the initial reduction of the metabolic rate is the same, but the cells exposed to ethanol continue to reduce their metabolic rate and eventually die. The cells exposed to DMSO recover their former metabolic rate within some hours and appear to make a full recovery. This finding has several implications. First, it implies that the mechanism by which the two compounds reduce cell metabolism is different. Second, the MRD_{50} is not sufficient to describe the

skin toxicity of a compound; information about recovery to exposure is also important. Clearly, ethanol is more dangerous than DMSO, even more so than the difference in MRD_{50} alone would indicate.

All work has been done on skin cells up to now, yet many further possibilities can be contemplated. One is to devise a small set of different cell types that would be representative of most of the body. The exposure of all these cells at once to a compound would then give a much more complete toxicological picture. This would go some way toward simulating the effect of injecting a compound in an organism. It would also be a more severe screen, even for substances intended for application on the skin, because the different cell types would be sensitive to more distinct damage mechanisms.

The use of human cells is probably considerably superior to a number of methods that have recently appeared that involve bacteria. Bacteria are more convenient to use in many ways: they have a longer life than human skin cells and are less delicate to handle and to culture. However, they are prokaryotes, a considerably more primitive type of cell than those in multicellular organisms. Thus, they could represent a poor model for the irritancy of human skin cells. The differences from mammalian cells that make them more convenient to handle also make the results from them less reliable. A well-chosen array of human or mammalian cells would appear to be the best choice for accurate toxicity determinations.

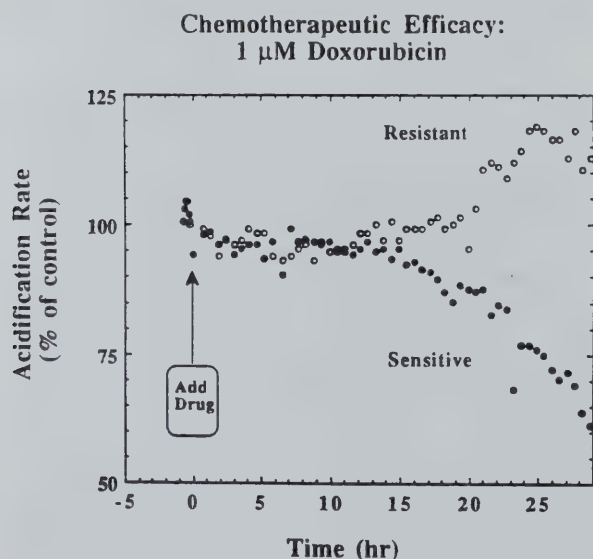


Figure 57. The metabolic rate is shown of two cell lines, one of which is resistant to a certain drug (doxorubicin), and one of which is not.

4.6.2 Determination of Chemotherapeutic Sensitivity of Tumor Cells One of the treatment methods used against malignant tumors is the administration of chemotherapeutic drugs. These drugs are cytotoxic, and the intent is to use them to eliminate the malignant cells without excessive damage to healthy cells. The correct selection of a drug (or combination of drugs) is a critical clinical decision, because tumor cells can be resistant to some of the drugs. At present, the decision of that drug to use is not based on testing the actual tumor cells of the patient. The reason is that existing bioassay methods to determine the sensitivity of tumor cells to chemotherapeutic agents depend on culturing the small number of cells retrieved by biopsy, and are too slow for clinical use.

The silicon microphysiometer can provide a more rapid bioassay that uses a very small number of cells. This enables information concerning the resistance or susceptibility to a drug to be gathered within about 24 hours. This could be rapid enough eventually to allow clinical use. Figure 57 shows an example. The metabolic rate is shown of two cell lines, one of that is resistant to a certain drug (doxorubicin), and one that is not. The control cells, never exposed to a drug, have a slowly increasing metabolic rate that indicates growth. The metabolic rate of the drug-sensitive cells, when exposed to doxorubicin, stops growing and then declines. The drug-resistant cells are not affected. It is interesting to note, however, that even the cells resistant to doxorubicin are sensitive to another drug, cisplatin. Bioassays along these lines can therefore be helpful in selecting the drug to which a given tumor is sensitive.

This information by itself may not be sufficient, however. A typical clinical problem in chemotherapy is the development of resistance to the drug used. This may be caused by selection of tumor cells: if a few cells are drug resistant, they will soon proliferate at the expense of the others. Therefore, a chemotherapeutic agent needs to be selected that is able to kill all the tumor cells. Often, this will require several drugs used in combination. It is not clear whether a bioassay is possible that can be of assistance in understanding and fighting the phenomenon of the development of resistance to chemotherapeutic agents. Certainly, it should be possible to place cells in a microphysiometer, expose them to a sufficient dose of a certain drug, observe the reduction in metabolic rate, and then wait for possible resistant strains to take over and cause the metabolic rate to increase again. The problem may be that this takes too long to be of use clinically.

Viral Infection and Inhibition by Ribavirin

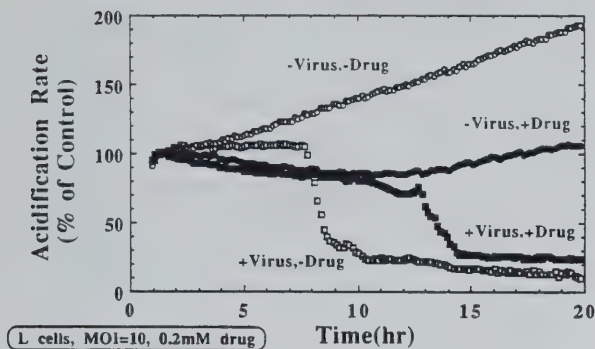


Figure 58. Effect of viral infection with VSV where the virus is VSV (vesicular stomatitis virus), which afflicts cattle. The metabolic rate increases with time, until the effects of the infection cause a decrease.

4.6.3 Effect of Ligand/Receptor Interactions on the Metabolic Rate A large amount of work on discovering new drugs centers on finding ligands that bind to specific receptors. In this way, a drug can affect a desired physiological function with fewer side effects. The usual method of detecting whether a substance binds to a receptor is first to label a known ligand radioactively, and bind it to all receptor sites. The cells with the receptors and labeled ligands are then exposed to a variety of substances under test. If a certain compound is able to displace some of the radioactivity, then it binds to the receptor. Aside from the use of radioactivity, this procedure has the disadvantage that it does not distinguish between agonists (that trigger a given response), and antagonists, that bind to a site but do not trigger a response.

Using the microphysiometer, it has been shown that it is possible to rapidly detect changes in metabolic rate caused by receptor ligand binding [105–108]. The effect of agonists and antagonists can be distinguished and quantitated.

4.6.4 Medium Optimization One straightforward application that comes to mind naturally is to use the real-time metabolic rate measurement for optimizing media for tissue culture and cell culture. The aim here is to find media that optimize the metabolic rate of a given cell line. This is slow and tedious if no real-time feedback on the cell's activity is available. With a microphysiometer, the effects of the addition or the removal of a constituent become visible in real time. Thus, the time needed in an empirical search for the optimal medium that meets a given set of requirements can be shortened considerably.

4.6.5 Applications on the Detection and Identification of Bacteria In this application, the usual procedure of looking at a roughly constant number of cells and doing something that changes their metabolic rate is not followed; instead, the metabolic rate of the cells is constant, but their number is allowed to grow. Provided sufficient nutrients are available, the number of bacteria in a given medium will grow exponentially. According to eq (49), their metabolic rate will be proportional to their number, and will also grow exponentially. By measuring the metabolic rate as a function of time, we can measure the rate of the exponential increase, that is usually expressed as a doubling time.

We have found doubling times that correspond well to the literature values. This opens up the possibility of identifying bacteria in this way. Another tool for identification is adding an antibiotic to the medium stream and measuring its effect. Given the knowledge of the spectrum of an antibiotic, more identification information can be gathered in this fashion.

4.6.6 Detection and Inhibition of Viral Infections In the case of viruses, a cell line of infected host cells is needed to culture the virus. The spreading of the virus can then reduce the metabolic rate [104]. An example is shown in figure 58, where the virus is VSV (vesicular stomatitis virus), that afflicts cattle, and the cells are L cells. The metabolic rate increases with time, until the effects of the infection cause a decrease. The degree of infection can be observed with an optimal microscope. For uninfected cells, the metabolic rate keeps on increasing due to proliferation.

Adding Ribavirin, an antiviral drug, inhibits at least partially the decay of metabolic rate of infected cells. It does not harm the uninfected cells, although they do proliferate less than the control cells.

The application here is the search for antiviral drugs. By adding various compounds to the basic culture medium flowing over the cells, compounds can perhaps be found that inhibit the spread of these viruses.

4.7 Summary

The silicon microphysiometer may well become the most important application of silicon chemical sensor technology in the area of chemistry and biology. It takes advantage of two attributes of silicon: very flat and small chemical-sensing areas can be made in it, and it can be micromachined to form accurate miniature flow chambers and cavities for

cell entrapment. Alternative technologies can probably accomplish each of these aims individually, but the combination of the two is unique. Applications in that these strengths are exploited will be the ones in that silicon chemical sensor technology reaches large markets in large numbers.

5. References

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Market Introduction Issues for Chemical Microsensors

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Chemical sensing has long been an established method for measuring and monitoring chemical species in a large number of media and environments. Recognition that semiconductor- and integrated circuit-based technology can be applied to chemical sensors created great excitement among sensor researchers, manufacturers, and users. This realization occurred more than two decades ago and resulted in projections that silicon-based sensors, such as chemical field effect transistors (CHEMFET) and ion-sensitive field effect transistors (ISFET), will revolutionize the chemical sensors industry similarly to the way microprocessors revolutionized the computer industry. In reality, this revolution has been slow to start. Today, it is possible to say that the chemical sensors industry is starting to feel the revolutionary impact of these technologies. There are still only a handful of silicon-based chemical microsensors on the market.

Commercialization of chemical microsensors has been much more difficult than researchers anticipated. A number of issues and barriers have impeded their rapid commercialization. Among these, the most important have been an underestimation of the effort required to apply silicon-based and microfabrication approaches to chemical sensors, lack of expertise to manage the multidisciplinary development nature of sensor-

based products, and slow adaptation to the changing nature and competitive structure of the sensor market. Despite these issues, the sensor industry has shown remarkable progress in understanding and resolving a number of fundamental technological limitations. It is anticipated that during the next decade a large number of new chemical microsensor-based products will appear on the market. Their success will be based not only on technological solutions, but also on better matching of applications to sensor capabilities and performance characteristics.

The biomedical applications, such as blood-chemistry monitoring and infectious-disease detection, and industrial applications, such as environmental monitoring and on-line process control, remain the most attractive applications for chemical sensors. The potential market size for these applications is large and can expand further if the chemical microsensors realize the cost/performance characteristics that the new materials and microfabrication technologies can offer.

Key words: biomedical sensors; biosensors; blood-gas sensors; chemical microsensors; chemical sensors; chemical sensors markets; hazardous gases sensors; immunosensors; microsensors; process-control sensors; sensor commercialization.

1. Importance of Chemical Sensors

The history of the development of chemical sensors is relatively short but eventful. In fundamental terms, chemical sensing provides the means to detect, diagnose, and monitor the chemical composition and changes of a particular system or envi-

ronment. Another major part of sensor functionality is the appropriate and timely transfer of information in a form that can be used for decision making and response. Clearly, chemical sensors play a critical role in controlling most industrial

processes by monitoring natural and industrial environments and assisting in the diagnosis of humans and animals.

Chemical microsensors are receiving significant attention today for several reasons. A reduction in the cost of computers and microprocessors has created a great demand for distributed intelligence closer to the event that requires monitoring, which provides better control. This demand has resulted in an explosive need for the robust and timely information that sensors provide as the front end of measurement and control systems. New technologies in sensor design and fabrication are capable of building highly sensitive, selective, and reliable sensors at low cost. These new sensor technologies and their greater capabilities offer the opportunity to address new markets for on-demand, on-site measurements and diagnostics. Bedside monitoring at hospitals, patient diagnostics in physician offices, and monitoring critical points in industrial processes are examples of such applications. The capabilities of distributed measurements, enhanced by distributed intelligence and better decision making, lead to significant improvements in product quality and customer service. These improvements directly affect the bottom line of a company and provide sustainable competitive advantage.

Chemical sensors are typically classified according to the state of the material they sense. Dr. Wen Ko defines three types of chemical sensors: gas sensors, ion sensors, and molecular sensors [1]. Biosensors are chemical sensors that use or sense biomedical materials.

Gas sensors monitor gases in:

- Environmental air pollution, such as NO₂, CO, CO₂, H₂, SO₂, H₂S, etc.
- Industrial operations, such as H₂, Cl₂, HCN, NH₃, O₂, humidity, gasoline vapor, fuel/air ratio, etc.
- Home environment, such as natural gases, odors, CO, etc.

Ion sensors monitor or measure the ionic concentration in an electrolyte or a solution. The sensors can also be used to measure pH in industrial or biomedical solutions or to measure a variety of other important ions, such as Li⁺, Na⁺, K⁺, Ca⁺⁺, etc.

Molecular sensors and biosensors are typically a combination of a molecular-recognition part and a transducer part. The molecular recognition part takes advantage of biological materials known for their specificity to certain molecules such as, enzymes and antibodies. The transducer can be

chemical sensors such as electrodes for pH, H₂O₂, NH₃, O₂; physical sensors detecting light, heat, sound, etc.; or a microchemical sensor such as IS-FET. Examples of such sensors include:

- Enzyme sensor for glucose using an O₂ or H₂O₂ electrode
- Enzyme sensor for cholesterol using H₂O₂ electrode
- Enzyme sensor for urea using a pH electrode
- Microbial sensor for CH₄ using an O₂ electrode
- Enzyme immunosensor for albumin using an O₂ electrode
- Immunosensor for therapeutic drugs such as theophylline and digoxine using antibodies and Ag/AgCl electrode
- Immunosensor for infectious diseases using antibodies and Ag/AgCl electrode.

Combining these sensor principles with the emerging application of microfabrication technologies has opened the door for the development of a new generation of microsensor-based products that will offer improved performance and price characteristics. Based on the last decade of sensor research, it is now possible to start anticipating the concrete results that will emerge on the market.

Given the possibilities in chemical sensing and the opportunities offered by new sensor technologies, new materials, and new fabrication techniques, the next decade is expected to be rich in producing a new generation of advanced chemical sensor- and microsensor-based products for a wide variety of applications. However, it should not be assumed that the path to new sensor commercialization will be easy. This paper will attempt to highlight some of the issues related to chemical-sensor commercialization and will address the most likely and attractive areas for sensor applications.

2. Commercialization Issues

There is going to be continued progress and innovation in the sensor field during the foreseeable future. The focus will be on improving present sensor concepts and systemizing sensor systems. Improving sensor concepts will come from the application of better sensing materials and advanced microfabrication technologies for miniaturization and energy savings. Systemization will be driven by an increased ability to integrate several sensing devices with various characteristics and signal-processing power. In this area, microfabrica-

tion- and microprocessor-based computing will play a significant role. The major areas of anticipated improvement and advances in sensors include:

- Transducer design and technology
- Multiple sensor techniques
- Biorecognition element (e.g., sensing membrane) preparation and stabilizing techniques for biosensors
- New sensors (new concepts and measurement items or transducer concepts)
- Signal processing techniques and sensor systems.

Despite the promising developments in the last two decades and the clear benefits offered by better sensors, sensor commercialization has not been easy. While many of the “classical” chemical sensors are currently on the market, practically no true biosensors or chemical microsensors are in wide use today. Although many researchers, companies, and commercialization organizations have promised to produce chemical microsensors for a while, the reality is that few of these products have been produced. The difficulty of microsensor commercialization lies as much in the technology and the production of the final product as it does in the structure and the capability of the commercializing organization. The major factors and issues that impact sensor commercialization are discussed below.

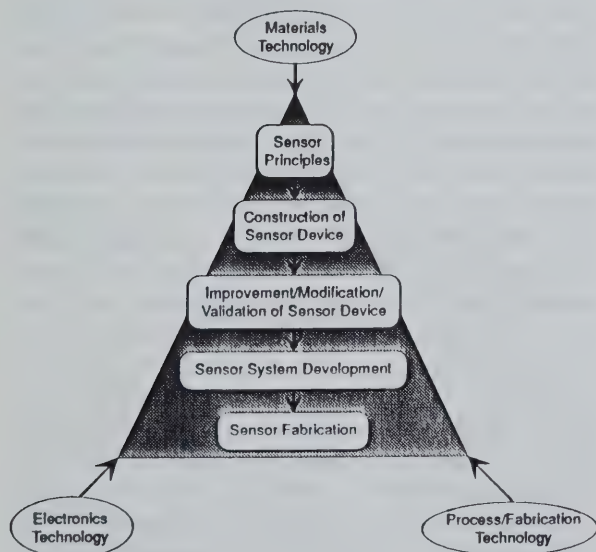


Figure 59. Sensor development and production process.

2.1 Technological Issues

Microsensor development is technologically driven. Developing a chemical microsensor today requires capabilities, skills, and an understanding of numerous technical fields. Figure 59 shows a schematic of the sensor development and production process.

While most of the principles required to develop sensor products are known, developing chemical microsensors still requires significant research effort. This is especially true in the area of fabrication. Microsensors have greatly benefited from the development of improved microfabrication technologies by the semiconductor and integrated-circuit industries. In many cases, this technology cannot be directly applied to microsensor manufacturing, because it needs to be adapted to the requirements of sensor manufacturing.

Applying integrated circuits (IC-) based microfabrication techniques, using silicon and other substrates, to chemical and physical microsensors has long been considered an ideal way to develop a new generation of better and less-expensive microsensors. Although this has been accomplished successfully for some physical sensors such as pressure sensors, in most cases, successful application to chemical microsensors has been difficult. While the benefits of microfabrication are clear, the assumption that the transfer of IC microfabrication technologies to chemical sensors is simple, has proved not to be the case. Still, significant problems need to be solved, and additional research needs to be conducted. Specifically, the major areas of fabrication incompatibility, between IC processes and microsensor processes, include:

- **Materials:** With the exception of few organics, most materials used for IC fabrication are inorganic. Chemical microsensors, on the other hand, require numerous organic materials to be incorporated in the manufacturing process, such as polyimide, hydrogels, membranes, and polymers. These materials can contaminate the processing equipment and affect the delicate processing conditions. Equipment duplication for different materials is likely to increase the capital cost for the sensor manufacturing process. Chemical sensors also require a greater variety of inorganic materials, such as Al_2O_3 , LaF_3 , AgCl , TiO_2 , Ir , IrO_x , etc. The IC industry is relatively inexperienced about the processing conditions for many of these materials. So additional research is required to fully understand how to consistently work with these materials.

- **Processing and Deposition Techniques:** With the variety of new materials used in sensor manufacturing, researchers often find that very little, if any, IC processing knowledge and experience can be transferred to help them in using these new materials. Thus quite often new processes and new methods need to be developed in order to avoid problems with:

- Processing temperature mismatch between organic and inorganic materials
- Adhesion to a variety of surfaces and materials
- Unknown materials characteristics due to new processing conditions
- Poor bulk material uniformity and surface activity
- Equipment contamination and lack of reproducibility.

- **Patterning Techniques:** IC patterning processes such as photolithography, screen printing, lift-off, and shadow masking do not work as reliably for sensors as they do for IC products. Often these processes either do not work with the materials that the sensor needs, such as membranes and hydrogels, or the level of deposition control is not satisfactory for chemical microsensors' geometries [2].

The impact of these issues has been one of the most serious impediments to producing commercially acceptable chemical microsensors. While there has been great progress in overcoming many of the above-mentioned barriers, using underdeveloped processes for the wrong applications can add significant cost, delays, and yield loss to any sensor development project.

Understanding the present stage of development of chemical microsensors may explain the dynamics of the situation and its evolution. Starting with a classical product life-cycle curve shown in figure 60, chemical microsensors can be shown to be still in early stages of development—the technology-driven period of the product life cycle.

Every emerging technology passes through an early period during which a dynamic interchange occurs between the new technology developers and the marketplace. The developers demonstrate the technology's potential, and the marketplace responds. In a form of feedback loop, this interchange occurs several times before customers and developers begin to understand which functional characteristics of the technology are most important and which characteristics offer best cost/benefit potential. During this period the developers

continue to improve the technology and manufacturing methods. By the end of this period both groups should be able to focus on the key issues and technical hurdles that must be overcome. This sets the stage for a higher volume of sales due to greater acceptance of the products using the new technology and a transition to the next phase of product-cycle development.

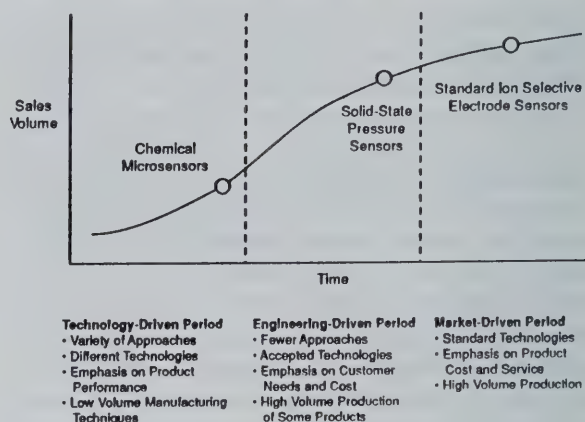


Figure 60. Life cycle evolution of sensor products.

At present, chemical microsensors are in the midst of this early dynamic interchange. The outcome of this interchange will be a clearer picture of what the technical priorities must be for the R&D community that will lead to faster commercialization of chemical microsensor products. Some of the results are already becoming clear. For example, although everyone agrees that eventual close integration between sensor function and signal-processing electronics will be extremely beneficial, the experience with CHEMFET shows that this type of extreme integration could slow down commercialization when applied prematurely. Until manufacturing technologies are developed to make such integration practical, it is more prudent for sensor manufacturers to design sensor systems that use proven fabrication methods for faster market introduction.

Knowing where a particular technology or product is on the life-cycle curve will help a developer understand how to select appropriate applications and customers, how to deal with potential customers, and how to focus on their needs. This view will assure the best match between sensor technology and appropriate application, thus increasing the probability of successful commercialization and product acceptance by the marketplace. As new chemical sensor technologies

become commercialized and production costs are reduced, individual and discrete sensors will become competitive with currently accepted techniques. This will lead to a broader acceptance of the sensor technologies, leading to a corresponding increase in sales volume. As volume grows, manufacturing economies of scale will improve and sensor cost will drop further.

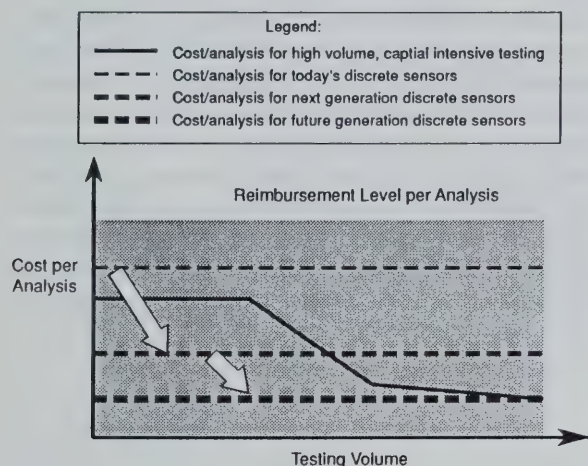


Figure 61. Generic structure of a sensor system.

Figure 61 demonstrates the impact of this market and technology dynamics for biomedical diagnostics. The figure schematically compares cost per analysis between discrete sensors and currently used high-volume, high-capital cost diagnostic equipment installed at hospitals and various large private laboratories. As testing volume increases, cost per analysis for the high-capital-equipment methods drops significantly, while the cost for discrete sensors remains the same. Presently, discrete sensors are more expensive for high-volume labs to use than existing approaches. Therefore, their profitability potential is limited, assuming both methods are equally reimbursed. However, as new and lower-cost sensor technologies are developed, cost per analysis for discrete sensors will be reduced, thus increasing their profit potential. The improved profitability potential and sensor ease of use, combined with multiparameter sensors and timelier access to information inherently provided by sensors, lead to a very compelling reason for laboratories and individual physicians to switch to this diagnostic method. This trend in economic and user benefits, offered by the new generation of chemical microsensors, is persuading numerous large, small, and venture capital-backed companies to invest in significant technical and financial re-

sources, with the goal of delivering the first sensor-based products to the market.

2.2 Organizational Issues

Organizational requirements also play an important role in the commercialization of new technology such as chemical microsensors. Chemical-microsensor development is truly a multidisciplinary effort. Figure 62 shows a generic structure of a microsensor system and highlights the technologies that may be used in a complete sensor-based system, from the chemical-sensing element to the electronic signal-processing system. Managing the variety of skills necessary at each stage of development is a tremendous challenge—not only because of the number of people and the diversity of skills required, but also because scientists and engineers are often not accustomed to work outside their own disciplines. This challenge is being addressed somewhat by the sensor research organizations established by a number of well-known universities. These programs offer graduate students the opportunity to specialize in sensor technologies, thus becoming accustomed to the multidisciplinary nature of the technology. As more students graduate from these programs, the required skills will become adopted by the industry. It is then up to the managers to recognize the diversity and breadth of skills required to develop chemical microsensors and to allocate the appropriate management and capital resources.

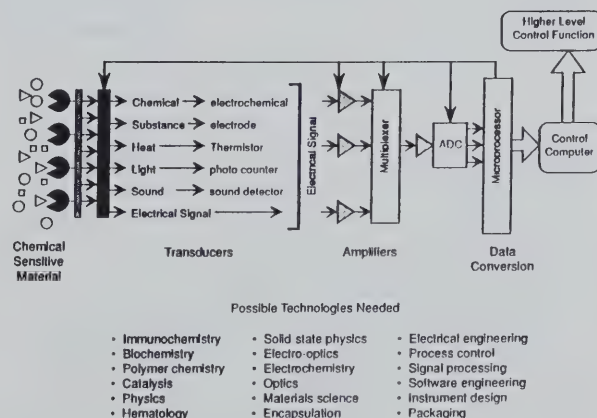


Figure 62. Economic barriers to acceptance of discrete sensors versus high volume, high capital cost diagnostic equipment.

2.3 Product-Introduction Issues

The third—and possibly most important—factor affecting chemical microsensor commercialization

is the extremely diverse and segmented chemical sensor market. Figure 63 is a partial list of potential application areas for chemical sensors. A key characteristic of the sensor marketplace includes not only the large number of sensors, but also the difficulty of transferring technologies and sensor products from one application to another. Often a completely new development effort must be undertaken to address a new market segment. Most of the effort goes into sensor packaging and interfacing with the new environment. This sometimes is the costliest aspect of the R&D and manufacturing development effort.

In addition, different markets require different selling and distribution channels. For a single company to tackle more than one or two of these segments is very difficult. In fact this difficulty has been the prime cause of chemical sensor companies' lack of significant growth. Traditional ways of looking at a business do not apply to the sensor business. Only a few companies will succeed in

growing to a significant size by considering themselves "sensor companies." On the whole, such companies will remain small with limited development resources. To build the critical mass required to develop chemical microsensors, successful companies have the option of either focusing on a few well-defined market segments of significant size to justify the required development expenses, or to become well-integrated solution companies offering a total solution to their customers. In this case, the sensor becomes the factor differentiating a company from other competitors and providing a sustainable competitive advantage. It is similar to selling the hole rather than the drill to customers who need the hole for their operation. The latter strategy offers a greater opportunity for success, since there are only few well-defined sensor market segments that are large enough to justify the resources required to develop a novel chemical microsensor.

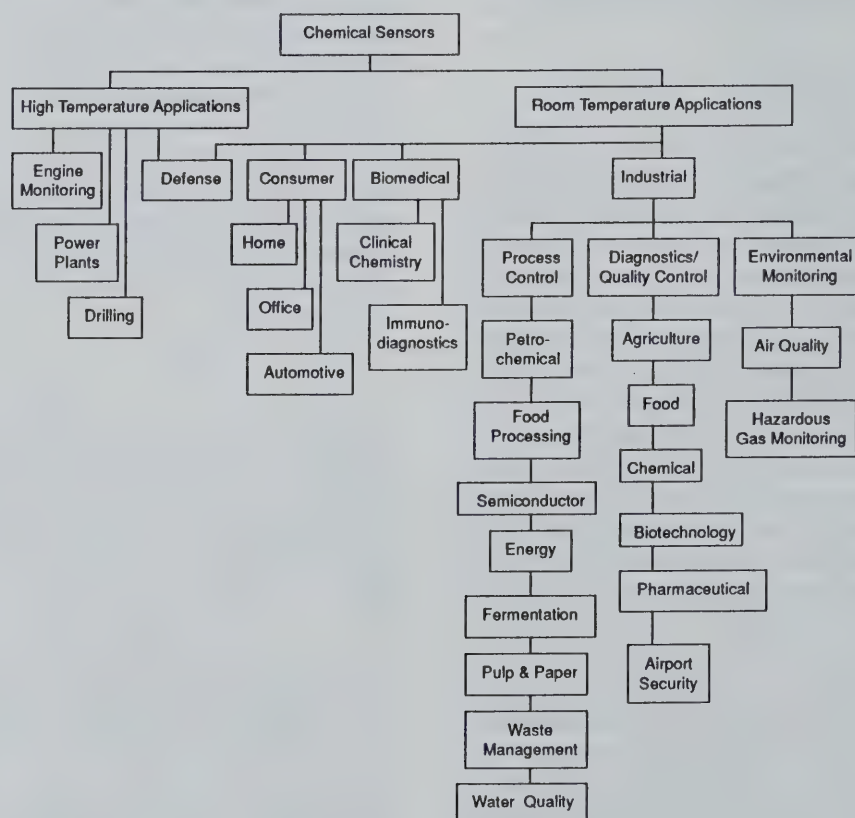


Figure 63. Chemical sensor applications.

As microsensor technology matures, it will become easier to transfer technology between sensor applications and to develop cost-effective products for diverse applications. Broad sensor capabilities can then be built and used as a basis for growing a significant sensor company. For the near term, companies that offer total measurement solutions, diagnosis, or process-control solutions to a well-defined customer base can best exploit the sensor technologies as a way of differentiating themselves from competitors. By selling more expensive control systems than just sensors, these companies can generate the required capital for microsensor research and development.

It is possible to argue that a sensor business does not actually exist. Rather, sensor technology is an enabling technology that can be effectively used as a competitive tool to differentiate a company from its competitors when they sell control systems or diagnostic results. For example, Nellcor Corporation in Fremont, California, is a company that has grown to more than \$100 million in sales in 5 years by selling pulse oximeters to hospitals. The company's success was based not only on its early introduction of an easy-to-use and superior optical sensor to measure saturated-oxygen levels in blood, but on its emphasis to solve the hospital's need for an easy, inexpensive, and noninvasive monitoring of critically ill patients.

Similarly, Measurex Corporation in Redwood City, California, built its business by providing complete process-control solutions and better service to the pulp-and-paper industry. While the performance of its systems depended critically on high-quality sensors, superior sensor technology combined with an in-depth knowledge of the needs of the pulp-and-paper industry was used as a competitive weapon to sell multithousand dollar systems, rather than several-hundred-dollar sensors.

Figure 64 schematically represents how process-control companies such as Measurex, Foxboro, and others can gain a competitive advantage by using advanced sensor and software (i.e., intelligence) technologies. This figure also demonstrates that as a company moves up the value-added chain from sensors to complete process-control systems, its revenues and potential profitability will proportionately increase. A company supplying process-control systems sells higher-priced systems with greater perceived value by the customer than does a company supplying just sensors.

The following sections of this report focus on several specific chemical-sensor market segments

and applications. While the sensor-application markets constitute numerous segments within many different industries, there are a few large markets that offer the most near-term application opportunities for new sensor products. As mentioned above, some sensor-application areas can be more productively penetrated by companies focused on complete system solutions using advanced sensor-based systems.

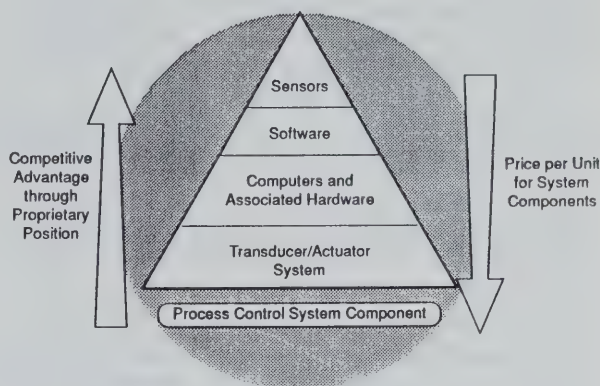


Figure 64. Strategic importance of sensors to process control systems companies.

3. Chemical-Sensor Applications and Markets

Chemical microsensors represent the continuation of a trend started several decades ago with the invention of ICs and the start of a new era driven by an explosion in information technologies and enormous improvements in the functionality of all industrial and consumer products. By starting with significant improvements in sensing concepts and new materials, chemical microsensors are now poised to be commercialized by taking advantage of the microfabrication technologies developed by the semiconductor industry. The challenge of sensor developers will be to apply the existing, and develop new, microfabrication capabilities that address the unique requirements for fabricating chemical sensors on a large scale. This challenge will require a careful match of sensor designs and configurations to the appropriate sensor applications. Table 12 lists in detail (beyond fig. 63 above), the expected applications and targets of chemical sensors.

It is clear that chemical sensor production will be divided into two types:

Table 12. Application of Chemical Sensors in Various Fields^a

Field	Sensor target	Aim
Consumer electronics and home appliances	Humidity, dew, turbidity Gas species, ionic species	Convenience, comfort, high quality, high function, safety, energy conservation
Automobile, etc.	Humidity, dew, smog, etc. Gas species, air-to-fuel ratio, etc.	Comfort, high quality, high function, safety, pollution control, energy conservation
Chemical and metallurgical industry (incl. petroleum, fermentation, foodstuff, etc.)	Solute components (organic, inorganic) pH, turbidity, ionic species, electrolytes solvent components, gas species	Process control, labor-saving security systems, pollution control, energy conservation
Labor environment (oil field, coal mine, ship, gas stand, underground, etc.)	Volatile solvent, gas species hazardous substances, etc.	Security systems, disaster prevention, fire prevention, industrial hygiene
Living environment, office environment	Humidity, gas species, fire, smog, etc.	Safety, comfort, smell, combustion control, energy savings, fire prevention, industrial hygiene
Health, medical care	Blood components, uric components, microorganisms, other bio-related compounds	Health management, diagnosis, medical engineering, remote diagnosis, treatment, closed-loop drug delivery, artificial organs
Agriculture, forestry, fisheries	Humidity, water content, freshness gas species, moisture, pH, electrolytes, bio-related compounds	Facilitate agriculture, cultivate fishery, freshness, delivery
Meteorology, oceanography station	humidity, salinity, turbidity, gas species	Unmanned observatory telemetry
Energy, nuclear power, natural resources	Gas species, metal ions, pH, humidity	Steam power plant control, combustion control, mines, energy conservation
Community in city	Gas species, smog, electrolytes or soluble substances, hazardous substances, pH, turbidity	Pollution control, disaster prevention, safety control, automatic monitoring

^a See reference [3].

- Mass production of widely used sensors for large monolithic market segments
- Small-scale production of special-purpose and custom-designed sensors for smaller, more diverse market segments.

Examples of the first type of sensor include oxygen sensors for automotive combustion control, sensors for smoke alarms and other consumer applications, commonly used blood-chemistry sensors for hospital applications, and home-diagnostic sensors for pregnancy, glucose, cholesterol, etc. Commercial production of such sensors will total in

millions of pieces per year. Cost-effective and high-quality production will be extremely important.

The second type of sensor will include a number of special-purpose sensors for the automatic control of various industrial processes in the chemical and fermentation industries, biomedical sensors that address specialized diagnostic needs such as cyclosporin™ monitoring for organ transplant patients, etc. These types of sensors require close integration with their own control system and the environment within which they will be functioning. Therefore, they will be custom-made to match the control and operation environment for which they

are designed. Understanding the needs and the applications for the intended sensor use will be critical to success in this area.

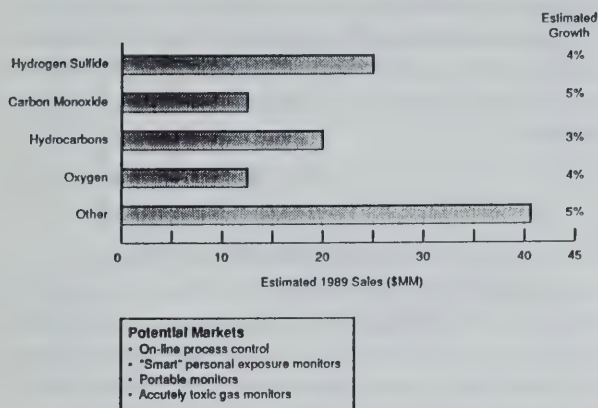


Figure 65. Representative markets for toxic and combustible gases.

Success in developing and marketing chemical microsensors will depend on an appropriate match between sensor technology, required development effort, cost to bring the sensor to market, and targeting a market segment that can provide satisfactory returns for the required investment.

3.1 Industrial Applications

Industrial applications for chemical microsensors represent potentially a large market opportunity. The key requirements for this marketplace are cost-effective and reliable sensors that integrate well with the appropriate operational environment.

Discrete sensor applications and toxic and combustible gas-monitoring markets are relatively established and will continue to provide large-volume opportunities for new products. Figure 65 shows

the estimated sizes of some market segments. The toxic and combustible gas-monitoring markets have a combined sales volume of \$100 million to \$125 million. The growth rate of these markets closely follow the capital expenditures in the industrial markets they serve. For example, in the case of hydrogen sulfide, growth is tied to the health and growth of the petrochemical industry. On average the growth is expected to stay below 7%, unless governments install additional or more stringent requirements for monitoring the industrial workplace.

Food-quality monitoring is another diagnostic area that has the potential for significant growth during the next decade. As food processing (e.g., meat and chicken-processing plants) becomes more automatic and the processing lines increase the speed of food handling, the likelihood of contamination will also increase. In the past decade, manufacturers have had to comply with legislation that requires new monitoring techniques and demands that products be made more cost-effectively and with consumers who are increasingly aware of food and nutritional quality. These trends will be driving the demand for better and easier-to-use sensors to detect possible contamination and to improve process control. Table 13 shows the estimated size of the world food-diagnostics market.

On-line process control is another highly attractive application for more specialized and custom chemical sensors. In this case, a sensor might provide a highly selective and sensitive front end to a control system that will use this information to monitor and modify an industrial process. With the development of a "smart" sensor, it is possible to envision, in the near future, greater use of control systems with distributed intelligence. These systems will offer better process control by monitoring more parameters and responding faster to process changes.

Table 13. Worldwide Food-Diagnostics Market (\$ Millions)^a

Test type	1986(\$)	Est. 1991(\$)	Annual growth
Salmonella	50	64	5%
E. Coli	25	31	4%
S. Aureus	20	26	5%
Yeasts	10	13	5%
Molds	10	13	5%
Listeria	10	20	15%
Campylobacter	5	8	10%
Others	10	13	5%
Market size	140	188	7%

^a See reference [4].

While sensors for on-line process control are extremely attractive, it is important to keep in mind that each specific application will probably require additional development effort. For example, a pH sensor for water-quality monitoring cannot be used for fermentation monitoring unless it is sterilized and will not be affected by interfering agents in the fermentation matrix. Additionally, very few industrial processes require microsensors. In most cases there are no space limitations for introducing a sensor in a process stream. Chemical microsensors, however, do make sense when the microfabrication technology can deliver multiple sensing capability on a small substrate, preferably with some intelligence closely associated with the sensor. In such situations, microfabrication can offer significant advantages in improved manufacturing economies of scale and superior price/performance characteristics.

One of the most interesting applications for on-line process control is the monitoring of fermentation and bioreactor-based processing. This application can cover many areas and industries, from beer production to drug manufacturing [5]. More specifically, it can include:

- Fuel-alcohol production
- Yeast production
- Production of organic acids (e.g., acetic acid, lactic acid, citric acid)
- Production of enzymes for detergents, cheese making, diagnostics, etc.
- Production of amino acids for dietary supplements (e.g., monosodium glutamate, aspartame, etc.) and medical antibiotics (e.g., penicillin, vaccines, steroids, etc.)

This is now a relatively young market. New sensor technologies open attractive application areas as they bring high value added information and allow better process control. The parameters that need to be monitored vary widely. They include temperature, pressure, agitation, foam production, pH, conductivity, redox potential, dissolved oxygen, dissolved carbon dioxide, effluent gases, and a variety of specific organic and inorganic molecules [6]. A monitoring system for bioprocess measurement faces significant challenges, because the sensors have to perform reliably and be cost-effective. From an operational perspective, sensors must perform in an environment that requires them to be sterilizable to avoid batch-to-batch contamination, and must they also withstand contamination by biological deposits inside a reactor. These are chal-

lenging problems that are addressed by using new materials and sensor concepts. Microfabrication techniques will lead to low-cost fabrication of multiparameter sensors that will make it cost-effective to use disposable sensors for each batch. As these new technologies are commercialized, the bioprocess control market may emerge as an important market for high value added, sensor-based control systems.

3.2 Biomedical Applications

In terms of market size and use, chemical microsensors for the biomedical field have a profound impact on human health-care management. During the last decade, health-care applications have been the most fertile area for advanced sensor research. Chemical sensors for biomedical use, such as biosensors, represent the possibility to combine three of the most revolutionary developments in the century: microelectronics, biotechnology, and microfabrication. The size of the biomedical market and its general willingness to use new technology and products represent the other reason why so much effort has been focused on this field.

While chemical sensors have great potential in the biomedical area, currently only a few applications are well established. For home and hospital use, one of the largest markets for sensors is monitoring blood glucose levels for diabetics. This market is roughly \$250 million per year in the United States, with a healthy growth rate of 8 to 10 percent. The majority of sensors used are based on colorimetric techniques. The recent introduction of a home-use enzyme sensor for glucose, by Medisense, Inc., represents the first consumer application of a true chemical sensor. As the benefits of this technology are recognized and as the cost per analysis drops, this technology will likely gain a greater share of this market.

For hospital applications, the most established application areas for chemical sensors are: transcutaneous sensors for carbon dioxide and oxygen monitoring; laboratory blood gas analysis for measuring blood pH, CO₂, O₂, electrolytes and other blood components; and extracorporeal and bedside blood-gas monitors. Figure 66 shows estimates of the market size for these products in the United States.

The transcutaneous monitoring market is quite mature, primarily addressing hospital neonatal and pediatric departments. Extracorporeal blood-gas monitors represent a relatively new market. These monitors measure pH, CO₂, and O₂ and are most

commonly used during open-heart surgery. Although the potential for growth in this market is significant, total growth is limited to the number of critical surgery procedures performed in hospitals. Using these types of monitors for bedside patient monitoring may significantly expand the market size.

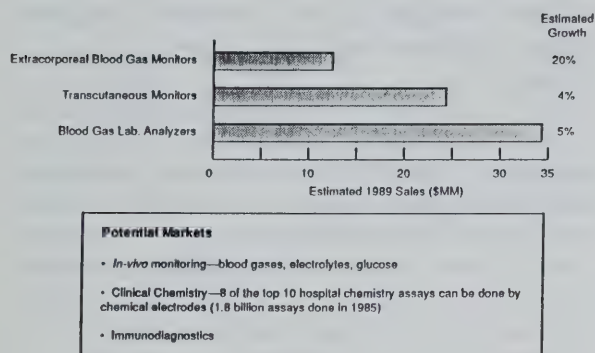


Figure 66. Representative markets for biomedical chemical sensors.

Figure 66 also lists some of the most attractive future markets for biomedical chemical microsensors. Of these, in-vivo sensors and in-vitro immunosensors for distributed, bedside, and alternate test-sites applications, promise an optimum match between sensor performance (e.g., biosensors), sensor cost characteristics, and the need of the marketplace. These areas are explored in more detail below.

3.2.1 In-vitro Immunodiagnostic Applications

The in-vitro diagnostics (IVD) marketplace is undergoing a transition from a centralized to an alternate site testing (AST). Customer needs in AST market segments differ significantly from the needs of conventional hospital and independent laboratories. Bedside and physician office testing, for example, demands simple, reliable, inexpensive instrumentation that is essentially self-calibrating and maintenance free. The preferred instrument is a small, multiparameter device capable of measuring a panel of analytes using an unmeasured droplet of blood from a finger-stick sample. Reagents must be entirely self-contained, factory-calibrated, safe, and disposable with prolonged shelf life. The test menu must be flexible enough to satisfy a variety of AST needs with a standardized, foolproof protocol. Results must be accurate, precise, rapid, and operator independent.

“Biosensors”—biological sensor elements (e.g., receptors, enzymes, antibodies) coupled to trans-

ducer devices (electrical, thermal, optical, etc.) to allow direct conversion of a biochemical parameter into an electronic signal—offer tremendous potential in meeting the needs of the AST market. The commercial potential of biosensors for in-vitro diagnostics, however, has not been realized because of technical difficulties. These difficulties are associated with:

- The efficient, stable coupling of the biological sensing element to the transducer device
- The amplification of the sensing event to yield adequate sensitivity for major market analytes (therapeutic drugs, hormones, disease markers).

Enzyme-linked immunosensors are being developed around principles derived from the enzyme immunoassay and enzyme electrode fields, as the most promising approaches to resolving these technical difficulties [7]. Most commonly, enzyme-labeled analytes and sample analytes compete for a limiting amount of antibody immobilized on the electrode surface. After the unbound enzyme-labeled analyte is removed by washing, the current generated by the bound enzyme-analyte conjugate is inversely proportional to the concentration of sample analyte. This approach harnesses the versatility and specificity of immunoassay and the sensitivity achievable through enzyme amplification. Ease of use, however, is inadequate for most AST applications. The need for a wash step to separate bound from free label introduces a source of error and operator dependence. New technologies, combined with efficient microfabrication technologies, are starting to address these problems. Within the next 2 years, it is expected that results from this research will reach the marketplace.

In 1988, the U.S. IVD market was \$3.5 billion. Industry analysts forecast that the U.S. IVD market will be approximately \$6.5 billion by 1995. Spurred by cost-cutting reimbursement policies diagnosis-related groups (DRGs), demographic trends toward an older population, and the availability of new advanced technologies, the fastest-growing segment is expected to be the decentralized (or ambulatory) IVD market segment. The ambulatory IVD market segment includes testing performed in physicians' offices, acute-care hospitals, outpatient facilities, nursing homes, and patient self-testing. This market is expected to reach over \$1.1 billion by 1995. The breakdown of IVD performed by location is shown in table 14.

The anticipated growth in the ambulatory and decentralized IVD market reflects significant

changes and trends in the health care system. These trends support and encourage increased testing at decentralized sites. Products that provide rapid, high-quality results and can be used by personnel without in-depth technical training in testing procedures are needed for this marketplace.

The product applications being considered by companies include the fast-growing areas of microbiological (\$460 million in 1988 and 10% growth per year) and immunodiagnostic (\$866 million in 1988 and 14% annual growth) testing. An example of a specific monitoring application is the monitoring of theophylline levels in asthmatic patients.

Theophylline is a drug used to treat more than 6 million asthmatic and allergic adults and children worldwide. Regular monitoring is necessary to assure effectiveness and avoid toxicity. The 1989 worldwide market for theophylline testing is about \$120 million (the United States represents more than one-half this market). Other interesting potential applications are shown in table 15.

3.2.2 Invasive Blood-Gas Monitoring Another example of a large and highly attractive market for

biomedical microsensors is continuous in-vivo monitoring of blood gases, electrolytes, and pressure [8]. Of these, the in-vivo blood-gas-monitoring market represents the one with the greatest potential. To address this need requires the development of a sensor product that consists of a disposable probe. This probe should measure pH, O₂, and CO₂ and fit easily through a 20-gauge arterial catheter. The probe must also be able to operate reliably and accurately in a patient for at least 72 hours.

The continuous blood-gas-system market consists of two major components: the disposable blood-gas sensor probe, which can be used in one patient only and the monitoring instrument, which displays the information. The market for the disposable probe is \$360 million to \$540 million per year in the United States. For the monitoring instrument, there is a potential to install 10,000 to 20,000 units in the United States.

Although the in-vivo blood-gas-monitoring market is attractive, it has been difficult to crack for many companies. There are two competing techni-

Table 14. U.S. in vitro diagnostics market by laboratory segment, 1986–1990 (\$Millions)

	1986	1988	1990
Centralized sites:			
Commercial Laboratories	\$829	\$896	\$929
Hospitals	1,842	2,105	2,345
Decentralized sites:			
Clinics/HMOs	154	210	283
Physicians' offices	154	175	242
Patient self-testing	92	140	242
TOTAL	\$3,071	\$3,506	\$4,041

SOURCE: Biomedical Business International

Table 15. Representative examples of U.S. IVD immunoassay markets (\$Million)

	1987	1990
THERAPEUTIC DRUG MONITORING (TDM)		
Theophylline	\$51	\$65
Digoxin	\$38	\$45
THYROIDS		
TSH, T4, Tu	\$55	\$65
OVULATION/FERTILITY	\$15	\$20
INFECTIOUS DISEASES	\$550	\$670
TOTAL	\$709	\$865

cal approaches for measuring blood gases in-vivo: fiber-optic-based sensors and electrochemical-based microsensors. The most serious problem experienced by these technologies has been to achieve reliable sensor operation in the blood [9]. For some of the measured parameters, a number of technologies have demonstrated reliable operation for up to 48 hours. But the users such as physicians have been reluctant to accept products that partially satisfy their needs. The extremely large market potential and ability to reliably combine microelectronics, sensor chemistry, and microfabrication will make this application the pinnacle of success for a new generation of chemical sensors. Within the next 2 to 5 years, this application will demonstrate the great promise that chemical microsensors will fulfill.

4. Conclusion

Various types of chemical microsensors are making remarkable progress. Multiple private and public-sponsored multidisciplinary efforts are addressing sensor materials, concepts, and manufacturing issues. Most significant research is focused on practical issues that will lead to rapid development of practical and economically viable sensor-based products. Although there is still great hope for the development of "smart" sensors using silicon chip technologies, investigations of FET-based sensors have demonstrated how difficult it is to make such sensors for various operating media. To be successful in this area, a company must carefully evaluate the value of its sensor technology or concept, clearly understand what level of capital and effort will be required to produce the sensor, and determine if the targeted application and market for this product will provide an acceptable return on investment. An optimum match between customer needs, required sensor-performance characteristics, the ability to satisfy these characteristics with a particular sensor concept, cost and effort of development, and market size will guarantee that a sensor R&D effort will be completed in a timely manner and that the product is readily accepted by the market.

The flow of new ideas, concepts, and multidisciplinary research efforts will continue to keep the excitement in this field high for the next decade. The potential for the chemical microsensor business can be compared now to the early stages of the application-specific integrated circuits (ASIC) business. Less than a decade ago, ASICs were only

feasible for extremely high volume or high value-added applications. These applications could justify their high development cost. With development of sophisticated computer-aided design (CAD) tools and gate arrays, the development and manufacturing costs suddenly dropped to such an extent that now it is difficult to find a product using electricity that does not use one or more ASIC devices. ASIC production and development is now a multi billion-dollar industry. The increased multidisciplinary sensor research activity can be expected to achieve the same effect. Over time, better design tools, more or less standardized chemical sensor building blocks, and low cost manufacturing processes will allow numerous sensor-based products to be economically introduced, thus creating a dynamic billion dollar chemical sensor industry.

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